

Real-Time MR Imaging of Stem Cell Delivery to the Central Nervous System of Small and Large Animal Models using EPI

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Target audience: Physicians and scientists interested in stem cell-based therapy and ultra-fast real-time imaging.

Purpose: Tremendous progress in the field of stem cells has been recognized this year by Noble Prize committee with the award to prof. Yamanaka for the discovery of induced pluripotent stem cells, potentially unlimited source of highly therapeutic stem cells. For the success of cell-based therapy it is of utmost importance to develop effective and safe cell delivery methods and that in turn highly dependent on cellular imaging for non-invasive monitoring of the process of cell delivery. Up until now, MRI stem cell tracking has only enabled detection of cells after the transplantation was completed. We have developed a new technique that allows monitoring of cell delivery in real-time, allowing immediate intervention would cells engraft in undesired locations including the formation of life-threatening microembolisms.

Materials and Methods: *Cell labeling:* Human mesenchymal stem cells (hMSC, PT-2501, Lonza) were treated overnight with 20 µg/ml of Molday ION-Rhodamine B (BioPAL, Inc.). Immediately prior to transplantation, cells were harvested and suspended in 10 mM PBS, pH=7.4 at 0.2-1.0x10⁶ cells/ml. Intraarterial cell transplantation was performed in four different experimental models: rat stroke model, normal porcine brain, normal dog brain, and normal dog spinal cord. For stroked rats, the animals were subjected to a carotid artery cannulation and hMSCs were administered via the internal carotid artery (n=20). For cerebral injections in dogs (n=2) and pigs (n=6), catheters were introduced under C-arm fluoroscopy via the femoral artery into the internal carotid artery. For assessment of cerebral perfusion, the labeled cell injection in the dog brain was preceded by infusion of the USPIO formulation Feraheme®. For spinal delivery in dogs, cells were injected in the Adamkiewicz artery, a relatively large anterior spinal artery found in humans and dogs but not in other species including pigs. *Imaging:* For real-time monitoring of Feraheme and labeled cell injection, we used a standard EPI sequence. For rats, a Bruker 7T horizontal bore magnet and 15 mm planar surface coil was used with TE=17 ms, TR=2000 ms, FOV=260x260mm, matrix=96x96, and acquisition time=2 s. For dogs and pigs, a 3T Siemens Trio was used with TE=36 ms, TR=3000 ms, FOV=1080, matrix=128, and acquisition time=3 s. These fast sequences enable continuous monitoring of cell delivery. Standard T2-w and susceptibility weighted images were also acquired before and after EPI.

Results: In the rat stroke model, we detected in real-time an inflow of cells into the brain characterized by a gradual, focal decrease of pixel intensities (PI) on consecutive images over a period of 600 seconds (Fig. 1A, infarcted area outlined in yellow). Based on PI, graphs were generated depicting the reduction of PI for selected ROIs. No change of signal was observed in the contralateral hemisphere. Real-time EPI demonstrated that cells rapidly engrafted within the stroke periphery, with a delayed inflow into the core of the infarct. At the end of the infusion, the cell distribution within the overall infarcted area was quite homogenous. The images in Fig. 1A are representative scans demonstrating the dynamics of cell injection and homing. The successful implementation of real-time imaging of cell delivery in rodents prompted us to test this further in a clinically relevant setting using large animals and clinical instrumentation. In dogs for spinal injections cells were infused into the Adamkiewicz artery and then monitored with EPI demonstrating their broad distribution within the lumbar and thoracic spinal cord (Fig. 1B). We detected and quantified the speed and magnitude of cell inflow to the different segments of spinal cord (Fig. 1B) showing more robust engraftment in proximal to Adamkiewicz artery ROI1 and 2, compared to distal ROI3 and 4. SWI scans typically used for detection of iron labeled cells were highly affected by motion artifacts and of low quality. Perfusion imaging using Feraheme® (three bolus injections of 3mg/ml, 300µl each completed in interval between 0 and 170s) was performed to predict the cell inflow area and preservation of cerebral blood flow (CBF) following cell delivery. Three separate bolus injections resulted in a dramatic, transient drop of PI in specific regions of the ipsilateral hemisphere (Fig. 1C, performed at 30, 80 and 120s). Cell injection completed in an interval between 170-400s resulted in a gradual PI decrease in the region previously highlighted by Feraheme® injection. To confirm that the CBF was not altered by transplanted cells, Feraheme® was injected a second time (i.e., after cell delivery, three boluses), demonstrating a similar perfusion in the area containing transplanted cells (Fig. 1C, interval between 400-600 sec.). In pigs, similar image guided cell delivery demonstrated efficiency of EPI for monitoring intracerebral cell engraftment and notably follow up MR imaging performed next day (n=3) and one week later (n=1) using T2-w sequence did not detect ischemia.

Discussion: Intraarterial injection is an attractive strategy for cell delivery. EPI enables monitoring of cell delivery in real-time with sufficient detail to evaluate cell engraftment and sufficient temporal resolution to discriminate early from late filling areas. Using traditional MRI T2* cell tracking techniques the interpretation of post-injection images was found to be problematic due other sources of hypointense signal. Dynamic EPI imaging of cells proved both more sensitive and more specific compared to SWI for spinal cord imaging.

Conclusions: We have shown that intraarterial cell delivery to the CNS can be monitored in real-time by MRI and that perfusion imaging with Feraheme® can be used as a predictor of cell distribution as well as a method for evaluating patency of CBF following stem cell injection. This method can be used to ensure a safe and efficient intraarterial delivery of stem cells in both small and large animal models, with the potential for clinical translation.

