IN VIVO MONITORING OF IMMUNE CELL KINETICS WITH TIME-LAPSE MRI IN THE ISCHEMIC LESION OF MOUSE BRAIN

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Target Audience: Researchers interested in *in-vivo* cellular tracking and neuro-immune interaction.

Introduction: *In vivo* imaging of immune cell recruitment into central nervous system (CNS) can help to understand the pathophysiological and molecular mechanisms of CNS inflammations and repair, and can provide significant diagnostic value for injury and diseases in the CNS, such as stroke and multiple sclerosis. We reported that 11.7 T MRI with *in-vivo* administration of superparamagnetic iron oxide particles (SPIO) has a possibility to visualize the recruitments and migrations of immune cells, especially phagocytes (monocytes or macrophages), in a mouse brain *in vivo*.[1,2] In addition, time-lapse movie analysis with 20 min/frame continuous scanning have a good feasibility to track and estimate the dynamic single-cell migration in the whole mouse brain noninvasively.[1,2] In this study, we improve the temporal resolution of time-lapse MRI movie with SPIO-based *in-vivo* cell labeling and assess the difference of velocity and direction of immune cell migration between the healthy brain and ischemic-injured brain.

Methods: SPIO (Resovist, 0.25 mmol Fe/kg body weight) were injected into the tail veins in each adult male C57BL/6 mice. At 12 hours after SPIO administration, T_2-weighted (T_2W) RARE and T_2^*-weighted (T_2^*W) FLASH sequences were taken as pre-surgery. Following scan, ischemic stroke was induced by a permanent middle cerebral artery ligation model (pMCAO).[3] Immediately after surgery, mice were subjected to diffusion-weighted MRI (DWI) and T_2W MRI to check the ischemic lesions. The mice that could not found any abnormalities in DWI within 1 hour after pMCAO were excluded from further experiments. At 24 hours after pMCAO (36 hours after SPIO administration), conventional T_2W MRI was taken to estimate the ischemic infarction (Fig.1A). In addition, continuous T_2^*W MRI measurements of whole brain with acquisition times of 5 minutes were taken for 6 hours (72 repetition) to allow for time-lapse MRI movie series (Fig.1B). T_2W hyperintensity lesion was overlaid on the T_2^*W series (Fig.1C), and the time-lapse 4D movie of each mouse brain was generated by the ImageJ 1.47v. The velocity and direction of labelled-cell migration were also measured by manual tracking plug-in (http://rsb.info.nih.gov/ij/plugins/track/track.html) in ImageJ 1.47v (Fig.1D). All animals were anesthetized with isoflurane and maintained a constant respiration rate of 80 ± 10 breathes/min during each MRI session. The mouse head was placed in a custom 15-mm inner diameter transmit/receive volume RF coil (m2m imaging). MRI was conducted on an 11.7 T vertical bore system (AVANCE II, Bruker). After the in-vivo MRI study, fixed brains of each mouse were stained with Hematoxylin & Eosin (HE) and Prussian blue (PB) to confirm the region specific SPIO accumulation in the mouse brain.

Results and Discussion: The accuracy of velocity and migration pattern was improved more than previous experiments (20min/frame) due to the shortening of frame rate of this study (5min/frame) (Fig.1B and 1C). We observed different migration patterns after permanent ischemia using time-lapse MRI (Fig.1D). In the normal control mice, the certain number of T_2^*W hypointense spots in the brain were moved along the blood vessels. The average velocity was 5.4 μm/min. The spots migrated basically from inside to outside and ran along the venous streams. These migration pattern was basically same in the contralateral side of ischemia group (Fig.1D left hemisphere). Meanwhile, the velocity of spots in the ischemic lesion was relatively slow (average 3.8 μm/min), and several spots migrated in opposite direction compared with normal condition (Fig.1D right hemisphere). In addition, several spots migrated orthogonal orientation to the blood vessel in the ischemic lesion. Although the most spots disappeared from the normal brain about 7 days after SPIO administration, the spots in the ischemic model mice remained long period in and around the infarct lesion. The spots gradually integrated around the infarct lesion. In addition, the T_2^*W-hypointensity area was reduced consistent with the timing of the T_2W-hyperintensity lesion elimination. The opposite direction of spots flowing from the brain surface toward the brain center appear associated with the perivascular glymphatic pathway[4]. This pathway may appear due to the artery occlusion. Thus, we speculated that the movement of the spots observed by time-lapse MRI during ischemia may have observed the kinetics of monocyte/macrophage recruitments that migrated along perivascular pathway. We confirmed ischemic lesion as pyknotic nuclei in histological sections stained by HE (Fig.1E) and the much higher number of SPIO pigmentations were observed in the lesion stained by PB than the those in the contralateral hemisphere (Fig.1F). Almost SPIO existed near the viable nuclei (Fig.1G), so that we speculated those viable cells were SPIO-labeled exogenous immune cells which transmigrated in the ischemic lesion.

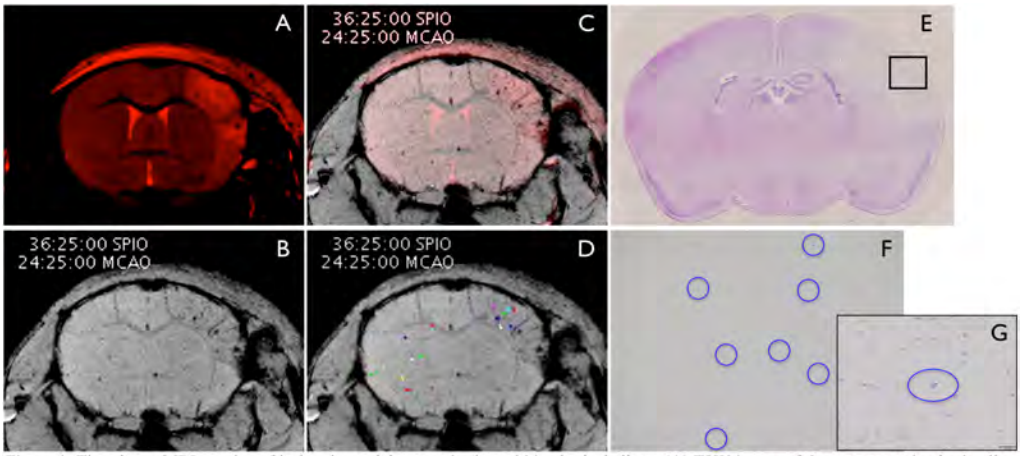

Figure 1. Time-lapse MRI movies of ischemia model mouse brain and histological slices. (A) T2W image of the representative brain slice shows hyperintensity ischemic lesion. (B) T2*W time-lapse movie of similar slice as A shows hypointensity spots that reflect the SPIO-labelled phagocytes migrating throughout the brain, and the spots abound in the ischemic lesion. (C) merged image with A and B. (D) trajectory of spots (colored dots and tail lines) in the ischemic lesion and contralateral side. (E) Representative HE-stained brain section shows the pyknosis in the ischemic lesion. (F) Prussian blue staining visualizes that the blue pigmentations reflecting the SPIO particles exist in the ischemic lesion (black square of E). (G) magnification of F. almost SPIO exist beside the nuclei (blue circle).

Conclusion: Time-lapse MRI have a good possibility to track the cell migration and assess the difference of cell kinetics between normal and ischemic lesion. MRI could be a new noninvasive cell tracking method and could bring about dynamic immune cell behaviours in disease progression or repair as well as in physiological conditions.

References: 1) Mori et al., 22nd ISMRM, 2013, #3813, Milan, Italy. 2) Mori et al., Sci Rep 2014; 4:6997, 3) Welsh et al., J Neurochem 1987; 49:846-851. 4) Nedergaard, Science 2013; 340:1529-1530.