SEQUENTIAL AND TIME-LAPSE MRI MONITORING OF PERIPHERAL MACROPHAGE RECRUITMENT AND MIGRATION IN MOUSE BRAIN

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Target Audience: Researchers interested in visualization of immune cell migration in central nervous system using iron oxide nanoparticles.

Purpose:

For more than a century, scientists have believed that the blood-brain barrier (BBB) is a sacred and impermeable wall, and that maintains the CNS homeostasis and protects the brain from harmful substances. However, recent studies demonstrated some immune cells, such as macrophages and microglia in the central nervous system (CNS) play an important role in the neuroinflammatory disease, such as multiple sclerosis [1,2] and ischemic injury [3]. The dynamical behavior of immune cells in intact/injured CNS has not been well characterized with *in-vivo* imaging techniques. Non-invasive monitoring of immune cells before/after injury may lead to a greater understanding of the mechanisms of both CNS inflammations and repair. We reported that MRI with superparamagnetic particles of iron oxide (SPIO) has a possibility to visualize the recruitments and migrations of immune cells in mouse brain *in vivo*.[4] We however have not yet characterized specific immune cells. In this study, we focused on peripheral macrophages and tested macrophage depletion methods to know whether macrophages are the main transporters of SPIO into the brain or not. In addition, we extend the use of MRI as a tool to monitor dynamic behaviors of cell migration in the brain with time-lapse MRI movie.

Methods:

All animals were anesthetized with isoflurane and maintained a constant respiration rate of 60 ± 10 breathes/min during each MRI session. The mouse head was placed in a 15-mm inner diameter transmit/receive volume RF coil (m2m imaging). MRI was conducted on an 11.7 T vertical bore imaging system (AVANCE II, Bruker). 1) Cell tracking & time-lapse MRI studies: The distribution of SPIO-labeled cells (endogenous phagocytes) in the mouse brain was checked in normal and LPS-induced systemic inflammatory conditions. SPIO (0.25 mmol Fe/kg body weight) were injected into the tail veins in each subject and T_2^*-weighted FLASH sequence was taken sequentially. Dynamic cellular tracking for an extended time period (24 hours) was demonstrated by sequential 2D images. 2) Selective cell depletion studies: To deplete the peripheral macrophages, clodronate liposome was injected into the tail vein two days before administration of SPIO. In addition, we synthesized stealth particles, which are not taken into any phagocytes, and checked the contrast of brain after injection of the particles. 3) Injured-model studies: T2-weighted and T2*-weighted MRI of artificial MCA-occluded model mice [5] were taken simultaneously, and tracked the cellular distribution around the ischemic lesions. After imaging, animals were sacrificed and brain sections were obtained for histological analysis.

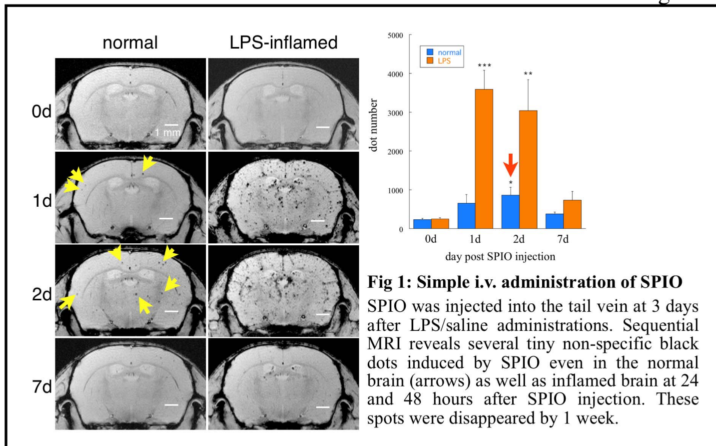

Fig 1: Simple i.v. administration of SPIO
SPIO was injected into the tail vein at 3 days after LPS/saline administrations. Sequential MRI reveals several tiny non-specific black dots induced by SPIO even in the normal brain (arrows) as well as inflamed brain at 24 and 48 hours after SPIO injection. These spots were disappeared by 1 week.

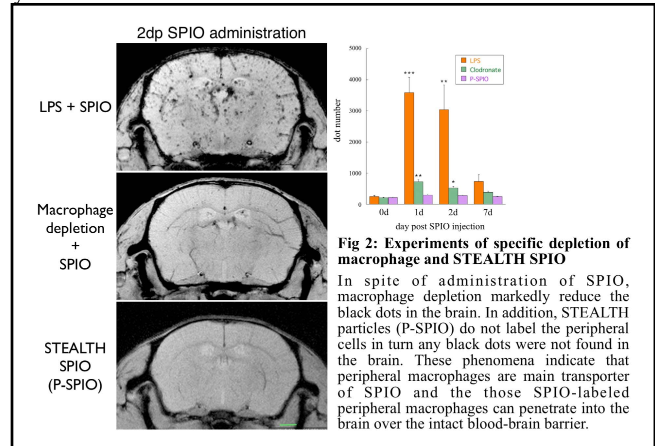

Fig 2: Experiments of specific depletion of macrophage and STEALTH SPIO
In spite of administration of SPIO, macrophage depletion markedly reduce the black dots in the brain. In addition, STEALTH particles (P-SPIO) do not label the peripheral cells in turn any black dots were not found in the brain. These phenomena indicate that peripheral macrophages are main transporter of SPIO and the those SPIO-labeled peripheral macrophages can penetrate into the brain over the intact blood-brain barrier.

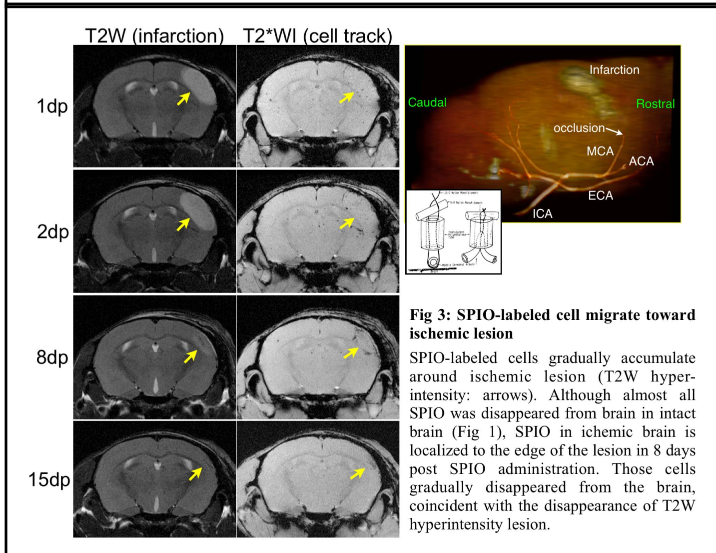

Fig 3: SPIO-labeled cell migrate toward ischemic lesion
SPIO-labeled cells gradually accumulate around ischemic lesion (T2W hyper-intensity: arrows). Although almost all SPIO was disappeared from brain in intact brain (Fig 1), SPIO in ichemic brain is localized to the edge of the lesion in 8 days post SPIO administration. Those cells gradually disappeared from the brain, coincident with the disappearance of T2W hyperintensity lesion.

Results and Discussion:

We observed several black dots even in the healthy brain at 24 and 48-hour post-SPIO administration as well as LPS-induced inflammatory condition (Fig.1). With selective macrophage depletion and histological analysis, we confirmed these black dots were SPIO-labeled endogenous peripheral macrophages recruited from blood (Fig.2). Improving temporal resolution carried out time-lapse MRI movie, and this movie can track single-cell migration in the whole mouse brain noninvasively. In the MCAO study, a lot of SPIO-labeled macrophages were detected around the infarction region. Some labeled cells were also found in the healthy area as same as we have seen in the control brain. Although most labeled cells in the healthy area disappeared by 1 week, cells aggregated at the edges of the infarction remained for a long time. Those cells disappeared from the brain, coincident with the disappearing of T2-weighted high-intensity region (Fig.3).

Conclusion:

MRI with intravenous SPIO injection can successfully monitor the recruitment of peripheral macrophages into CNS even in normal as well as abnormal condition. Time-lapse movie by MRI may reveal critical insights into cell behaviors that are not obtained by optical microscopy. Our technique could contribute to understand the neuro-immune crosstalk and to reveal the mechanisms of immune cell dynamics in the normal CNS as well as injuries, inflammation and diseases.

References: 1)Arima *et al*., Cell, 148: 447-457 (2012), 2) Mori *et al*., Int Immunol, epub ahead of print, doi:10.1093/intimm/dxt044 (2013), 3) Shichita *et al*., Nat Med, 18: 911-917 (2012), 4) Mori *et al*., Proc 20th ISMRM, Melbourne AUS (2012), 5) Welsh *et al*., J Neurochem, 49: 846-851 (1987)