Sequential and Time-lapse MRI Monitoring of Peripheral Macrophage Recruitment and Migration in Mouse Brain

Yuki Mori¹, Ting Chen¹, Koji Ohno¹, Shinichiro Yoshida¹, Yosiyuki Tago¹, Tetsuya Fujisawa¹, Yuuto Kashiwagi¹, Masaki Fukunaga², Yutaka Komai³, Yuutaka Hata⁴, and Yoshichika Yoshioka⁵

¹Biofunctional Imaging, Immunology Frontier Research Center (IFReC), Osaka University, Suita, Osaka, Japan, ²Center for Information and Neural Networks (CiNet), National Institute of Information and Communications Technology (NICT) and Osaka University, Suita, Osaka, Japan, ³Institute for Chemical Research, Kyoto University, Uji, Kyoto, Japan, ⁴Frontier Biochemical and Medical Research Laboratories, Kaneka Corporation, Takasago, Hyogo, Japan, ⁵Graduate School of Engineering, University of Hyogo, Himeji, Hyogo, Japan

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) was injected into the tail veins in each subject and T₂*-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 the tail veins in each subject and T₂-weighted 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) was injected into the tail veins in each subject and T₂*-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 the tail veins in each subject and T₂-weighted 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) was injected into the tail veins in each subject and T₂*-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 the tail veins in each subject and T₂-weighted imaging system (AVANCE II, Bruker).

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:
MRR 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.