HIGH-RESOLUTION MR LYMPHANGIOGRAPHY IN MICE USING DOUBLE CONTRAST AGENTS AT 11.7 T

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Introduction: Major advances in immunology have been gained using mouse models. However, few methods exist to visualize the lymphatic vessels of mice, since they are small in size, and the best of our knowledge, no methods can visualize the lymphatic vessels discriminated from concomitant veins. Divalent manganese ions are paramagnetic and greatly reduce the T_1 relaxation time [1], and also has a property as a low-molecular-formed contrast agent to make a contrast for the anatomical details on T_1-weighted images [2]. Manganese chloride ($MnCl_2$) solution administered interstitially enter not only the lymphatic capillaries but also the blood vessels easily due to their small molecular size, and allow the identification of the lymph drainage and blood vessels with MRI. We would like to point out that one of the major limitations of this technique is poor discrimination between lymphatic vessels and concomitant veins. We hypothesized that the drop in signal intensity in the blood vessels with negative contrast agents can discriminate and visualize the lymphatic structures clearly. Unsuccessful results have been reported in mice using double contrast agents injections. This study investigated to visualize the lymphatic structures of healthy mice on high-resolution three-dimensional manganese–enhanced MRI (3D-MEMRI) at 11.7 T. Furthermore, we try to discriminate lymphatic vessels from blood streams with simultaneous administration of $MnCl_2$ and intravenous injection of micron-sized particles of iron oxide (MPIOs).

Methods: Male C57BL6J mice were anesthetized with isoflurane in room air via a nose cone and maintained a constant respiration rate of 60 ± 10 breathes/min during each MRI session. Mice were placed in a 25-mm inner diameter transmit/receive volume RF coil. MRI was conducted on an 11.7 T vertical bore imaging system (AVANCE II, Bruker). Localization scans are used to ensure that the lower limb, popliteal, sacral and iliac lymph nodes are covered in the FOV. Following baseline scans, 3D-MEMRI was performed by intracutaneous 10 mM $MnCl_2$ remote injection (10 µL/min, 10 minutes) in the magnet with 3D-FLASH sequence (TR/TE = 15/1.8 ms). After that, mice were allowed to wake up and were placed back in their cage. With a 5-7-day interval, enough to clear contrast effects of first $MnCl_2$ administration, the mice were anesthetized again and received intravenous injection of 2.7-µm MPIOs induced outside of the MRI scanner. Mice and MRI cradle was reset in the magnet, and following baseline scans, 3D-MEMRI was performed similarly to the first MRI session. Images were reconstructed using ParaVision 5.1 (Bruker) and 3D MR images underwent maximum intensity projection (MIP) and multiplanar reconstruction (MPR) processing using Osirix DICOM viewer.

Results and Discussion: $MnCl_2$ diffused broadly toward the lymphatic drainage originating from footpad, so that popliteal, sacral, iliac and inguinal lymph nodes were delineated during and immediately after $MnCl_2$ solely administration. (Fig. 1a-c). Several tubes by way of each lymph node were found, although discrimination between lymphatic vessels and blood vessels was still difficult with $MnCl_2$ solely enhancement (Fig. 1a-c; white and red arrowheads). To resolve this limitation, we tested simultaneous administration of interstitial $MnCl_2$ as a positive contrast agent for lymphatic vessels and intravenous MPIOs as negative contrast for vein, respectively. Figure 1d-f shows the 3D-MIP/MPR images of a same mouse with intravenous MPIOs administration. MPIOs cannot leak from the blood vessels, because of their large size, thus they remain in the blood pool for several hours [3]. By virtue of MPIOs, lymphatic vessels were delineated clearly by $MnCl_2$ enhancement with exclusion of the signal from concomitant veins (Fig. 1d-f; white arrowheads).

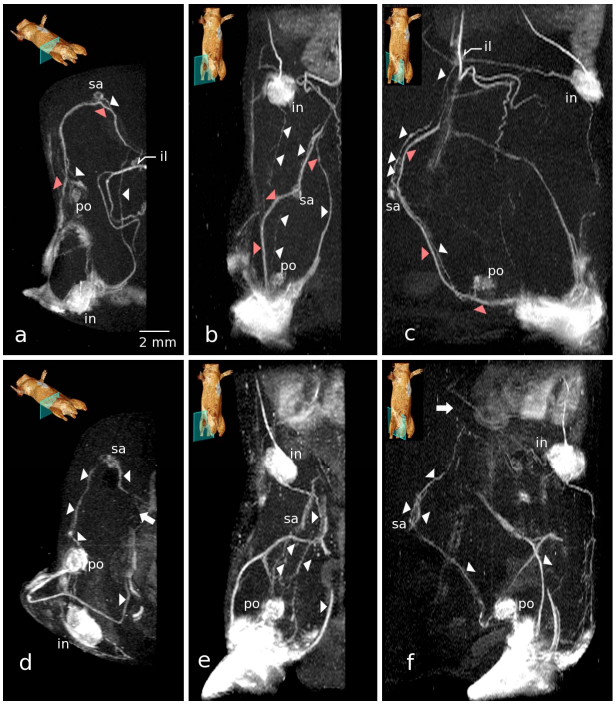

Figure 1. Combination of MPIOs and $MnCl_2$ injections can visualize lymphatic vessels separately. The above images were examined on same mouse in same session. MPIOs and $MnCl_2$ solution were combined to discriminate between lymphatic vessels and concomitant veins. A few tubes by way of lymph nodes were visualized before MPIOs administration (a-c, arrowheads). By virtue of MPIOs from a penile vein, signal intensity of vein (red arrowheads) was disappeared and lymphatic vessels were enhanced with $MnCl_2$ and discriminate from blood streams, separately (d-f; white arrowheads).

Conclusion: Our results demonstrate the detailed pathway of lymphatics in healthy mice with in vivo 3D MRI. Interstitial administration of $MnCl_2$ resulted in a fast and high accumulation in lymph nodes and depicted lymphatic vessels clearly. In addition, simultaneous administration of interstitial $MnCl_2$ and intravenous MPIOs discriminated lymphatic vessels from blood vessels. The findings also suggested that we need high-resolution images to get lymphatic anatomical informations specifically.

References: [1] Mendonça-Dias *et al*. Semin Nucl Med, 13: 364-76 (1983), [2] Massaad and Pautler. Methods Mol Biol 711: 145-74 (2011), [3] Decuzzi *et al*. J Control Release, 141: 320-7 (2010)