High-resolution interstitial MR lymphography for the diagnosis of sentinel lymph nodes: Inhomogeneous distribution of SPIO within non-malignant lymph nodes

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Introduction: Sentinel lymph nodes (SLNs) are lymph nodes (LNs) where lymph originating from a primary tumor flows into first, thereby resulting in lymphogenous metastasis. Since the presence of SLN metastasis predicts a poor prognosis, the differentiation between tumor-bearing, reactive (inflammatory) and normal SLNs is clinically important. Previous studies demonstrated the usefulness of MR lymphography for this purpose utilizing interstitially administered superparamagnetic iron oxide (SPIO) [1, 2]. In these studies, reactive and normal LNs typically showed homogeneous low signals on low-resolution MR images; however, compounds that are interstitially administered first reach marginal lymph sinuses at the periphery of LN, where afferent lymph ducts open and a large number of macrophages exist, and we therefore hypothesized that the accumulation of SPIO could be higher at the periphery than the center of LNs, leading to the inhomogeneous distribution of low signal intensity on T_2-weighted images (T_2WIs) of SLNs. The purpose of this study was to determine whether high-resolution MR lymphography could visualize the inhomogeneous distribution of low signals after the interstitial administration of SPIO using a lymphadenitis animal model.

Materials and methods: The experimental protocol used in this animal study was approved by the institutional animal experimental committee. All MR images were acquired using a 3−Tesla whole-body scanner (Signa HDx; GE Healthcare, Milwaukee, WI) and a dedicated RF coil for mouse imaging. Ten male ddY mice (7−8 weeks of age) were subcutaneously administered 0.05 mL Freund complete adjuvant into the left foot pad to produce lymphadenitis. The right foot served as a control. Two weeks later, six mice were administered 0.05 mL SPIO at a concentration of 3.3 μmol iron/mL into the bilateral foot pad (0.17 μmol Fe/foot). The concentration of SPIO accumulated in a 4-mm popliteal LN was presumably 0.25 μmol Fe/g tissue, assuming that 5% of the administered dose to the foot pad reached the popliteal LN [3]. The other 4 mice received the equivalent volume of normal saline (NS). Two hours after administration, T_2WIs of the popliteal LNs were acquired using a lipid-suppressed fast-spin-echo sequence (TR / TE_{eff} = 4000 / 60 ms, FOV = 4 cm, matrix = 256 * 192 [ZIP 512] yielding in-plane resolution of 78 * 78 μm^2, slice thickness = 1 mm, NEX = 2). In 3 SPIO-administered and 2 NS-administered mice, additional MR images were acquired 1 day after administration. Areas of high-signal intensity were measured on transverse *in vivo* T_2WIs through the center of the LNs. *Ex vivo* MR images of the excised and fixed LNs were also obtained. The area where iron-positive cells in the LNs were determined on Prussian blue-stained specimens was identified.

Results: Low signal areas were found in 11/12 popliteal LNs with SPIO administration on *in vivo* T_2WI. All inflammatory LNs showed peripheral low signals of various shapes, including rim-like, crescent, or wedge-shaped (Fig. 1). Spotty low signals were also seen in the central part in 3. The high intensity area in the inflammatory LNs 2 hours after SPIO administration was significantly smaller than after NS administration ($p = 0.001$, t-test). The high signal intensity was slightly reduced on day 1. *Ex vivo* MRI of the inflammatory LNs confirmed the peripheral low signals, except for one that showed homogeneous low signals throughout the whole area. Histologically, no cases showed large necrosis. Iron deposition was predominantly noted in the peripheral area within 200 μm from the capsule of LN in 4 cases (Fig. 2). A summary of the results is shown in the Table.

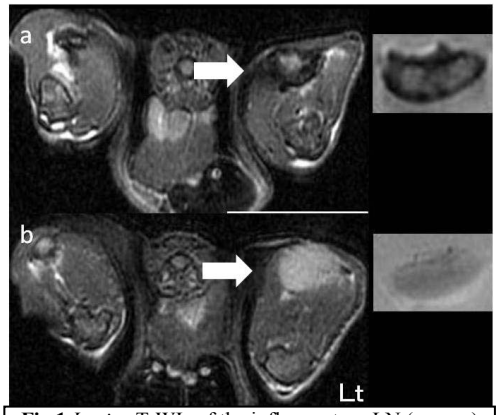

Fig.1 *In vivo* T_2WIs of the inflammatory LN (arrows) in SPIO-administered (a) and saline-administered (b) mice. Bars represent 10 mm. Insets are *ex vivo* MRIs of the corresponding LNs.

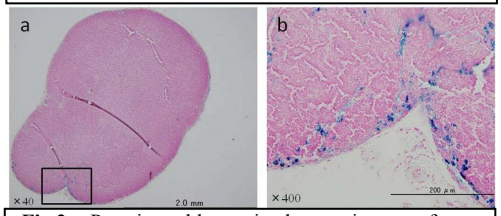

Fig.2 Prussian blue-stained specimen of an inflammatory LN at low (a) and high magnification. Iron-containing macrophages are displayed as blue dots in marginal and cortical perinodular sinuses.

Discussion: The distribution of low signals on T_2WI suggested that SPIO-laden macrophages were predominantly distributed at the periphery of inflammatory LNs. This finding was in accordance with our previous SPECT-MRI fusion imaging study of inflammatory LNs that demonstrated the peripheral distribution of ^{99m}Tc phytate, which was interstitially administered and phagocytosed by macrophages [4]. The Prussian blue-stained specimen suggested that the presence of SPIO-laden macrophages at the marginal sinuses rather than medullary sinuses possibly produced a peripheral low signal on T_2WI. Our results also suggested that SPIO-enhanced interstitial lymphography could display various patterns of signal distribution within inflammatory (reactive) LNs, as was shown on USPIO-enhanced intravenous MR lymphography [5]. High signal areas within SLNs visualized on SPIO-enhanced interstitial MR lymphography therefore do not always indicate the presence of metastatic deposits.

Conclusion: High-resolution MR lymphography could visualize the inhomogeneous distribution of interstitially administered SPIO within inflammatory and normal LNs.

References: [1] Radiology 1989; 171: 835–839 [2] Invest Radiol 1995; 30:706-711 [3] J Nucl Med 2001; 42: 951–959 [4] Proc ISMRM 2010; 18: 3963 [5] Radiographics 2009; 29: 1057−1069 **Acknowledgement:** This study was supported by a Grant-in-Aid for Scientific Research (KAKENHI) 22390240 from the Ministry of Education, Culture, Sports and Technology. The authors thank Mr. A Nabeteni and Mr. A. Nozaki for construction of the RF coil used in this study.

Table

	Contrast agent	*In vivo* MRI				*Ex vivo* MRI		Specimen	
		Area of high signal		Distribution of low signal		Distribution of low signal		Distribution of Fe**	
		2 hrs*	1 day*	Whole area	Periphery	Whole area	Periphery	Whole area	Periphery
Lt	SPIO	3.9 (3.2)†	2.8 (1.4)	0	6	1	5	2	4***
	Saline	11.3 (1.7)†	11.8	0	0	0	0	0	0
Rt	SPIO	NA	NA	2	3	2	4	1	5
	Saline	1.5 (0.1)	1.2	0	0	0	0	0	0

*Average area of high signal intensity in mm^2. SDs are shown in parentheses. In control group, the area could not be precisely assessed because the LNs were too small. **Hemosiderin, which is inherently present in LNs, was not included. *** Including one case of several Fe deposits at the periphery. † statistically significant ($p=0.001$, t-test)