Interstitial magnetic resonance lymphography using Gd-EOB-DTPA in VX2 rabbits

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Synopsis: The purpose of this study was to investigate the dose dependency of Gd-EOB-DTPA for interstitial magnetic resonance (MR) lymphography and the detection of lymph node metastasis in VX2 rabbits. The time course of signal to noise ratio (SNR) and contrast to noise ratio (CNR) between non-tumor region and metastasis in lymph node was examined and compared before and after subcutaneous administration of Gd-EOB-DTPA at 4, 8, or 17 micro mol Gd/kg. Our results suggest that that an interstitial MR lymphography with Gd-EOB-DTPA can potentially detect metastasis, and that the optimal dose in rabbits is 8 micro mol Gd/kg at subcutaneous application.

Methods:

Contrast agent: Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA: Schering AG) was used at the supplied concentration (0.25 mol/L).
Animal model: Eighteen male rabbits were anesthetized with xylazine hydrochloride and ketamine hydrochloride. They were inoculated with 0.5 mL of VX2 tumor cell suspension into the instep of the right hind limb. At 5 to 7 days after tumor implantation, all rabbits were subjected to a MR imaging study.
MR imaging: The rabbits were subjected to MR imaging study using a 1.0 T clinical imager (Magnetom, Harmony, SIEMENS). Prior to the administration of Gd-EOB-DTPA, MR images of the popliteal lymph nodes were examined with proton density- and T2-weighted turbo spin-echo sequence. Imaging parameters were as follows: TR/TE =3000/9.8, 59 msec (double echo), Matrix 256 x 186, one excitation, FOV 280 x 210 mm, 64 slices of 1.5 mm slice thickness. The echo train length was 7, and the acquisition time was 272 seconds. Gd-EOB-DTPA enhanced 3-dimensional T1 weighted spoiled gradient-echo images (FLASH; TR/TE = 6.1/2.6 msec, FA 30) were obtained before and at 2.5, 5, 7.5 and 10 min after administration with the following parameters: Matrix 384 x 288, FOV 280 x 210 mm. 96 slices of 1.0 mm slice thickness. The excitation number was 1. The acquisition time was 144 seconds. The rabbits were administered Gd-EOB-DTPA subcutaneously at a dose of 4, 8, or 17 micro mol Gd/kg into the foot pad, and a gentle massage of the injection site was performed for 2 minutes to improve lymph drainage, according to previous reports (1-2). After MR imaging, MIP images were obtained from 3D-T1 gradient-echo images. In addition, reconstruction images were obtained from PD-, T2- and T1WI, which was almost the same slice as the histological section.
Data Analysis: To assess dose- and time- dependency of enhancement by Gd-EOB-DTPA in the right popliteal lymph node, the signal intensities of the non-tumor regions in the lymph node and metastasis were measured. The signal-to-noise ratio (SNR) was calculated with the following equation: SNRnon-tumor=(SInon-tumor–background)/background, where SI is signal intensity. SNRmetastasis=(SImetastasis–background)/background. The contrast-to-noise ratio (CNR) between the metastasis and non-tumor region was calculated with the following equation: CNR=(SImetastasis–SInon-tumor)/background.
Statistical Analysis: Difference in SNR of the non-tumor region and CNR after the administration of Gd-EOB-DTPA at each dose was examined by Tukey test.
Dissection and Histology: After completion of the MRI, the right hind limb, including the popliteal lymph node, was removed. Subsequently, the hind limb was fixed in phosphate-buffered 10% formalin. The lymph node was then divided into two sections at the middle line, which was parallel with the plane of the femur and shin, and the sections were embedded in paraffin blocks. Sections from each paraffin block were stained with hematoxylin and eosin for histological examination.
Results and Discussion:

The typical images of the PD-, T2- and Gd-EOB-DTPA enhanced T1WI are shown in Figure 1. The popliteal lymph nodes were detected as a homogenous hyperintensed region in the PD- and T2-WI images (Figure 1). Compared to the histological findings, the contrast between the metastasis and non-tumor region was unclear on both images. Therefore, it was only possible to evaluate the size and shape of the lymph nodes using the PDWI and T2WI techniques.

Gd-EOB-DTPA enhanced T1WI showed a hypointensed region for the metastasis and a hyperintensed region for the non-tumor region at any dose (Figure 1). The signal intensity reached a maximum at 2.5 minutes after administration, and the signal enhancement tended to be maintained at a high dose (Figure 1). In histological findings, the metastasis was diffusely observed in some medullary sinuses (Figure 1). In all rabbits, edema was induced by the stenosis and embolization of the lymphatic capillary in the medullary sinus containing the metastasis, although the structure of the medullary sinus was morphologically unchanged. Consequently, signal enhancement with Gd-EOB-DTPA seems to be caused by the presence of lymphatic flow.

Regarding the quantitative signal evaluation, it was found that the SNR of the non-tumor region remarkably increased at 2.5 minutes after the administration of Gd-EOB-DTPA, and then gradually decreased at any dose (Figures 2A-C), whereas there was no change in the SNR of the metastasis (Figures 2A-C).

The SNR of the non-tumor region was shown to be dose-dependent: although the SNR of the 8 micro mol Gd/kg group was significantly higher than the 4 micro mol Gd/kg group ($p<0.01$ by Tukey test); there was no significant difference in the SNR between the 8 and 17 micro mol Gd/kg groups (Figure 3A). The CNR (shown as a minus value) between the metastasis and non-tumor region showed almost the same dose-dependent profile as the SNR of the non-tumor region (Figure 3B). Therefore, the optimal dose for contrast enhancement was 8 micro mol Gd/kg in rabbits.

In the MIP images reconstructed from T1WI, the structure of the lymphatic vessels and lymph nodes was visualized at any dose (Figure 4). There was a tendency for prolongation of signal enhancement at a higher dose. In addition, the signal of the bladder was gradually enhanced (Figure 4). It is supposed that Gd-EOB-DTPA subcutaneously administered is 1) drained into lymph vessels, 2) absorbed into the blood flow in the lymph nodes, 3) circulated in the blood, and 4) excreted via urine and bile.

References: (1) Ruehm SG, et al. Radiology. 2001; 218: 664-669.
(2) Hernorn CU, et al. J Magn reson Imaging. 2003; 18: 328-335.

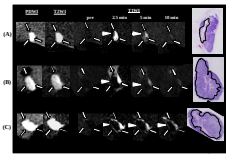

Figure 1. Correlation between histologic section with HE staining and PD-, T2-, and T1WI images using Gd-EOB-DTPA at a dose of 4 micro mol Gd/kg (A), 8 micro mol Gd/kg (B), or 17 micro mol Gd/kg (C). (arrows: right popliteal lymph node, arrow heads and inside of the line: VX2 metastasis.

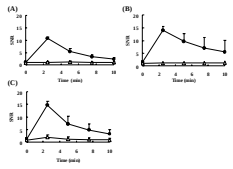

Figure 2. Time course of SNR in popliteal lymph node(non-tumor region) (●) and metastasis (Δ) after subcutaneous administration of Gd-EOB-DTPA at a dose of 4 micro mol Gd/kg (A), 8 micro mol Gd/kg (B), or 17 micro mol Gd/kg (C).

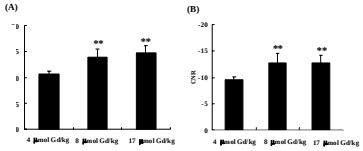

Figure 3. (A) Comparison of maximum SNR in non-tumor region among each dose. (B) Comparison of maximum CNR among each dose (n=6). **:$p<0.01$ versus 4 micro mol Gd/kg group by Tukey test.

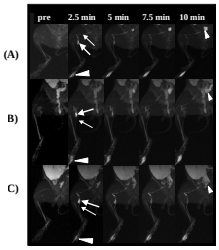

Figure 4. Maximum intensity projection images before and after subcutaneous administration of Gd-EOB-DTPA at a dose of 4 micro mol Gd/kg (A), 8 micro mol Gd/kg (B), and 17 micro mol Gd/kg (C). Popliteal lymph node (small arrows), lymphatic vessel (large arrows), the bladder (small arrow heads), and injection site (large arrow heads).