Potential of Newly Developed USPIO P904 in Detecting Lymph Node Metastasis

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Background

Recent analysis has shown that ultrasmall superparamagnetic iron oxide (USPIO) exemplified by ferumoxtran-10 (Sinerem) may detect focal cancer metastasis in the unswollen lymph nodes (1). ferumoxtran-10 has shown a good performance in clinical trial; however, currently unavailable. P904 (Guerbet Laboratories, Paris, France) is a newly developed USPIO. According to the preceding in-vitro data, the incubated macrophages have shown more rigorous phagocytosis of the agents, and in-vivo studies have shown more rapid clearance from the blood stream as compared with ferumoxtran-10 (2).

Purpose

The purpose of our study was to test the in-vivo performance of P904 in detecting lymph node metastasis, and assess the potential of the agent as a second generation USPIO after ferumoxtran-10.

Materials&Methods

The procedures were admitted by the internal review board (IRB) of our university and all the procedures were done under the guidelines for ethical conduct in the care and use of laboratory animals in our animal research laboratory. Five tumor bearing rabbits were used for the study. For preparation of lymph node metastasis model, 2.5 kg (BW) of male Japanese white rabbits (SLC Inc., Shizuoka, Japan) were subcutaneously inoculated with 0.5 mL of VX2 tumor cell suspension (1 X $10^{6\text{-}7}$ cells/mL) at the instep of right hind limb. At two weeks after tumor cell implantation, ipsilateral popliteal lymph nodes were used for metastatic lymph node model, and the contralateral side for control. Imagings were performed on 3.0T Signa HDXt (GEHC, WI, USA) with combined use of 8 ch knee coil under general anesthesia using 2.5% isoflurane inhalation (Escain, Mylan Inc., NYSE, USA). Following T1 weighted localizers, coronal 3D T2* weighted gradient echo images (3DT2*GRE) were performed covering bilateral popliteal regions. Parameters used were TR(ms)/TE(ms)/FA(degree) of 22/12/10, FOV (mm) of 120, 116 partitions with thickness (mm) of 0.7, NEX of 1 and total scan time was 11 min 19 s. 3DT2*GRE was chosen from four different contrast of optimized pulse sequences (i.e. 2D T2 weighted fast spine echo sequence (FSE), 2D proton density FSE, 3DT2*GRE, 3D T1 weighted GRE). The choice was made because of its optimum susceptibility effect and high spatial resolution. The 3DT2*GRE was performed prior to intravenous administration of P904 and 24 hrs after the administration. The injected dose was 100 micro mol Fe/kg. After the postcontrast MRI, animals were euthanized with excessive dose of intravenous administration of pentobarbital, and then, the bilateral popliteal lymph nodes were removed with marking of threads for orientation. The specimens were histopathologicaly examined by H&E, Perls/DAB (high sensitivity ion staining), anti-CK AE1/AE3 antibody immunohistochemical stain and macrophage staining. One experienced pathologist examined the ultrastructure of the lymph nodes with photomicroscope. Five point grading was made in terms of the areas of positive stain separately for the marginal sinus, the medulla, the cortex, the follicle and the metastatic foci of each lymph nodes. Anti-CK AE1/AE3 stain positive cell distribution was considered to be a standard of reference for metastasis in this study. For nodes with positive lymph node metastasis, signal of metastatic foci and non-metastatic foci were measured by placing a largest possiblt region of interests (ROIs) on the largest coronal section of each popliteal lymph nodes. Then, signal to noise ratio (SNR) and contrast to noise ratio (CNR) were calculated. The assessments were done by two investigators in consensus.

Results

Before contrast injection, there was no contrast between metastatic and non-metastatic foci; however, at 24 hours following p904 administration, the contrast between metastatic foci and non-metastatic foci was distinct; with CNR of 2.8 +/-0.3 (mean and SD) ($p < 0.0001$). The SNRs of the metastatic foci and non-metastatic foci were 4.19 +/- 0.34 and 0.03 +/- 0.05 respectively ($p < 0.0001$). The SNR of normal lymph nodes (contralateral side) was 0.06 +/- 0.06 ($p < 0.0001$). Histopathologically, the areas of signal loss were completely free from cancer cells, and were accompanied by various degrees of p904 distributions reflected by pearls DAB stain. The areas of remaining signal suffered from cancer cell infiltrations or dense lymphocyte infiltrations. SNR of the areas of reactive lymphocyte proliferations particularly at the follicles tended to be elevated even if cancer cells were absent. The densities of macrophage infiltrations were variable. They were densely accompanied by the margins of the metastatic foci and scattered at the medulla.

Conclusions

Newly developed USPIO P904 increased the contrast of metastatic foci in the rabbit lymph nodes at 3T.

References 1) Eur J Radiol. 2007 Sep;63(3):369-72. 2) Radiology. 2009 Aug;252(2):401-9.