Distinguishing of magnetically labeled cells from hemorrhage using positive contrast MRI

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Introduction

The labeled cells with superparamagnetic iron oxide (SPIO) can be traced using in vivo MRI. But the labeled cell and hemorrhage could not be clearly distinguished in conventional T2* weighted MRI. As iron storage protein, hemosiderin is always found within cells whereas ferritin is not only in the cells but also in the plasma. Hemosiderin is most commonly found in the macrophage, especially abundant nearby chronic hemorrhagic region. Although hemosiderin is a poorly understood protein, it is thought to be a complex protein of ferritin, denatured protein and other material. The intra-cellular aggregation of ferritin is believed to be the initial step in hemosiderin formation, and the boundary between ferritin and hemosiderin is not always clear. Since the molecular nature of the hemosiderin is not clear defined, we decided to use well-known ferritin for the hemorrhage mimicking situation.

The magnetization of ferritin powder is about 0.8 emu/g Fe (270 K)[1] and that of monocrystalline iron oxide nanocompounds (MION) is 63.8 emu/g Fe (293K) at 1.5T[2]. This difference could be used to distinguish ferritin from the labeled cell with SPIO with the positive contrast MRI method[3]. In this method, spectrally selective RF pulses are used for the off-resonance water surrounding the labeled cells. We try to distinguish the ferritin from the labeled cell with SPIO using the pulse sequence of the positive contrast MRI. This method could be applied to in vivo experiments.

Methods

MRI: The positive contrast MRI pulse sequence was made using EPIC and implemented on GE Signa 1.5T MRI, in which the signals were from the off-resonance proton excited selectively. The image parameters are following: FOV =12 or 8 cm, thickness = 4 mm and 512×512 or 256×256 matrix. Gradient echo with TR/TE = 300/ 9 or 11 msec, T2 weighted fast spin echo with TR = 3000 msec, effective TE =64 msec and ETL is 8. The positive contrast images were with TR/TE = 800/14 or 15 msec. **Simulated Hemorrhage**: The chronic state of hemorrhage lots of hemosiderin and ferritin were appeared. Horse spleen ferritin (F4503; Sigma Chemical, St. Louis, MO lot.084K7001) was used as a simulated hemorrhage. **Labeling**: Feridex (Berlex Laboratories, Inc, Wayne, NJ) was used to label the cell. **Cell**: To compare ferritin and Feridex property, we use peritoneal macrophage which was harvested in mouse (BALB/c 6-10 weeks). **Transfection**: Macrophage was incubated in 25 or 50 ug Fe/ml ferritin and Feridex solution for 18 hrs. *In vivo* imaging: The labeled macrophages with ferritin or Feridex, unlabeled macrophage (2.5×10^4 cells/ul, a half million cells) were injected into the hind limb of a live mouse**.**

Results

Ferritin and Feridex solution phantoms in test tubes consisted of the same concentration of iron (11.1, 5.55, 2.78, 1.39 and 0.69 mg Fe/ml). The high concentration ferritin solution show signal dephasing on the gradient echo image but do not show any dephasing effect in off-resonance image (Fig. 1). In contrast, Feridex solution tubes reveals dephasing effect on both gradient echo and off-resonance images.

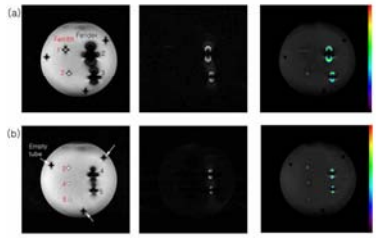

Fig.1. Phantoms of the ferritin and Feridex solution tubes with the high concentration solution (a) and the low concentration solution (b). The higher concentration ferritin solution tubes show signal dephasing on the gradient echo image (left) but do not show in off-resonance image (+1000 and +900 Hz respectively, mid). The right line is overlay off-resonance image on the spin echo image.

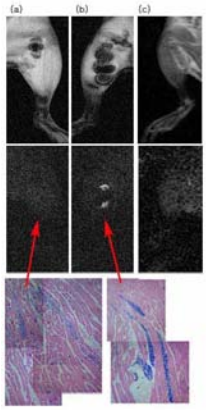

Fig.2. MR images with simulated hemorrhage (ferritin-macrophage, **a**), labeled cell (Feridex -macrophage, **b**) and unlabeled cell, **c**). The half million cells incubated in 25 ug Fe/ml (a,b) solution were injected into the hind limb of mouse. Ferritin-macrophage and Feridex-macrophage show signal decrease on gradient echo images (upper line, a,b). The unlabeled macrophage did not show signal decrease on FSE (upper line, c). Only the Feridex-macrophage recognize on the positive contrast image (+700 Hz off-set, mid line b). The images were cropped. The ferritin and Feridex were confirmed by Prussian blue stain (bottom line).

The positive contrast *in vivo* image recognized Feridex-macrophage but did not ferritin-macrophage (Fig. 2). As macrophages were incubated in the same concentration of iron, 25 ug Fe/ml, in ferritin or Feridex solution, iron concentrations were different, which was measured by ICP-MS (Feridex-macrophage and ferritin-macrophage, 9.1 and 5.3 pg Fe/cell respectively). Ferritin-macrophage was incubated in higher concentration of Fe solution (50 ug Fe/ml), but it could not be recognized on positive contrast *in vivo* image either (data not shown).

Discussion

The positive contrast imaging method could distinguish SPIO from ferritin even if they were consisted of the same concentration of iron. The labeled cells were recognized on off-resonance image injected into the limb of mouse, but the simulated hemorrhage did not. This method is anticipated to distinguish labeled cells from hemorrhage *in vivo*.

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