SUB-5 NM ULTRAFINE IRON OXIDE NANOPARTICLES FOR TUMOR IMAGING: NOVEL CONTRAST AND IMPROVED TUMOR UPTAKE

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INTRODUCTION

Superparamagnetic iron oxide nanoparticles (IONP) have gathered considerable attention for the development of MRI contrast agents and imaging-drug delivery in recent years. Although IONPs can navigate through the physiological barrier and accumulate in the diseased tissue, such as a tumor, via Enhanced Permeability and Retention (EPR) effect associated with tumor angiogenesis. Smaller nanoparticles (e.g., less than 12 nm) are favorable to penetrate leaky vessels for imaging of tumors (1, 2). Previous studies reported the synthesis of sub-5 nm ultrafine iron oxide (UFIO) nanoparticles that offer T_1 weighted contrast for vascular imaging and novel T_1-T_2 contrast switching in the different medium environments (3, 4). In this work, sub-5 nm UFIO nanoparticles (core size 3.5 nm) were used for imaging of tumor xenograft in mice and demonstrated capability of visualizing tumor vasculature and improved tumor uptake.

MATERIALS AND METHODS

Preparation of Nanoparticles and 4T1 Breast Cancer Mouse Model: Oligosaccharide coated sub-5 nm UFIO nanoparticles with a core size of 3.5 nm were prepared as previously reported and characterized by the transmission electronic microscopy (TEM) dynamic light scattering (DLS). To compare the particle with different sizes, 10 nm and 20 nm IONPs were made with the same method and the same coating. Prepared UFIO nanoparticles are highly stable in water for months with r_1 and r_2 of 4.1 and 16.4 at 3T, respectively. To make a mouse model of breast cancer, a total of 1×10^6 of 4T1 mouse mammary tumor cells were inoculated subcutaneously in the each side of upper mammary fat pads of 4 to 6-week-old female Balb/c mice. After 10-14 days post-inoculation, nine tumor bearing mice were randomly divided into three groups with each group administered with IONPs at one core size.

MRI Data Collection and Data Analysis: All MRI experiments were performed on a 3 Tesla MR scanner (Tim Trio, Siemens, Erlangen, Germany) using a phased array volumetric wrist coil. Mice were imaged before, 15 min, 2 h, and 24 h after the tail vein injection of IONPs (20 mg Fe/kg). MR imaging sequences included T_1-weighted spin echo (SE) imaging (TR/TE=500/10 ms, number of averages = 4), and T_2-weighted SE imaging (TR/TE=3200/65 ms, number of averages = 3), FOV = 60 × 120 mm^2, slice thickness = 1 mm. Additionally, a 3D GRE acquisition ultrashort echo time (UTE) imaging was applied in this study. The parameters for UTE imaging includes an ultra-short TE of 0.07, TR = 11 ms, flip angle = 10°, image matrix = 192 × 192 × 192, FOV = 159 × 159 × 159 mm^3, leading to an isotropic voxel size = 0.83 × 0.83 × 0.83 mm^3, bandwidth = 965 Hz/Pixel, number of readout samples in one radial projection = 384. Regions of interest (ROI) in the tumor were drawn manually on T_2-weighted SE images, including slices covering the whole tumor. The mean signal intensity from all ROIs were calculated using ImageJ (National Institutes of Health, Bethesda, MD, USA) for comparing the changes of signal intensity before and after injection of IONPs with different sizes. All tumors were excised after experiment and Prussian blue staining was used for confirming the IONP accumulation in tumors. Chemical analysis of iron was also performed to examine the levels of nanoparticle uptake by the tumors.

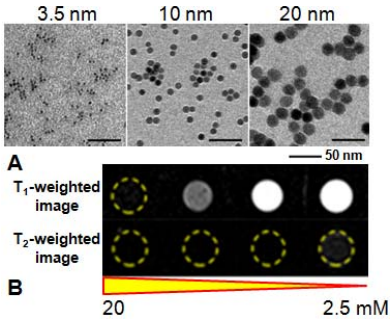

Fig 1. TEM images of UFIO and IONPs with different sizes (A). Sub-5 nm UFIO nanoparticles showed both T_1 and T_2 contrast on T_1 and T_2 weighted images (B) at 3 T.

RESULTS AND DISCUSSION

TEM images showed that sub-5 nm UFIO nanoparticles with an averaged core diameter of 3.5 nm were highly dispersed (average, **Fig. 1A**). They are size uniformed as those IONPs with core sizes of 10 nm and 20 nm. Sub-5 nm UFIO exhibited concentration dependent transition from T_1 to T_2 contrast effects when imaging with T_1 and T_2 weighted spin echo sequences (**Fig. 1B),** i.e. the signal increase on T_1-weighted images in the lower concentration range, but signal drop gradually on T_2 weighted spin echo images, when concentrations increased. After injection of sub-5 nm UFIO nanoparticles in the tumor bearing mice, the peripheral regions of tumors exhibited immediate signal increase on T_1 weighted spin echo imaging (15 minutes, as shown in **Fig. 2**). The bright T_1 contrast in the region was further enhanced and last beyond 2 hours after the injection of UFIO nanoparticles. However, 24 h after the injection, signals in the areas with initial T_1 contrast enhancement diminished. Instead, hypointense T_2 weighted contrast was observed in the various parts of the tumor, suggesting the accumulation of nanoparticles in the tumor tissue over time. The transition from T_1 contrast to T_2 contrast in the tumor likely indicates that UFIO nanoparticles leaked through tumor vasculature after the early phase of the delivery and later clustered in the tumor tissue upon accumulation. The presence of UFIO in the tumor and resulted T_2 contrast were further validated by the observation of bright or "positive contrast" in UTE imaging of the same tumor (arrow indicated in **Fig. 2F**).

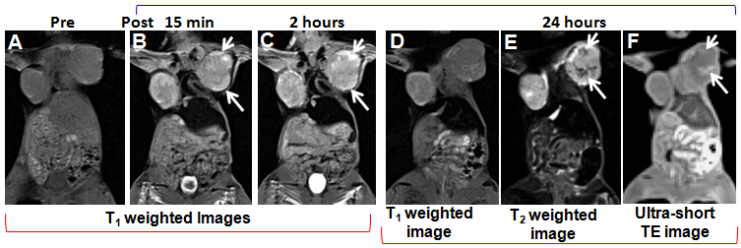

Fig 2. T_1 weighted spin echo images collected at 15 min (B), 2 h (C), and 24 h (D) after i.v. injection of UFIO nanoparticles showT1 contrast enhancement in the peripheral areas of tumors, comparing to pre-contrast image (A). Signal drop was observed in the tumor in T_2 weighted SE image (E) 24 h after the injection while the same region shown positive contrast in UTE image (F) due to the accumulation of UFIO nanoparticles.

To evaluate the effect of nanoparticle size on the particle penetration and accumulation in the tumor, the signal decrease and contrast changes in T_2-weighted SE images were compared between the groups received IONP with different sizes. It was found that the signal intensity change in the tumor at 24 hours post-injection was the greatest in mice injected with sub-5 nm UFIO nanoparticles compared to mice injected with IONPs with core sizes of 10 nm and 20 nm. The averaged signal intensity decreases in the ROIs of T_2 weighted images selected from tumors are 15%, 10% and 8% for mice injected with IONPs of 3.5 nm, 10 nm and 20, respectively (**Fig. 3A**). Prussian blue staining for iron further confirmed the high level of tumor uptake of UFIO nanoparticles (**Fig. 3B**). These observations suggest that the penetration and accumulation of IONPs in tumor tissue is likely size dependent and sub-5 nm UFIO nanoparticles are more efficient to be delivered to the xenograft tumor.

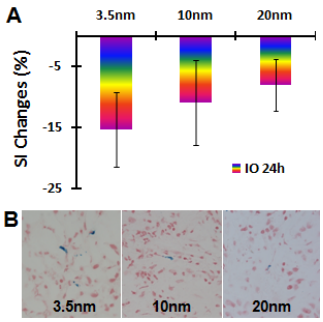

Fig 3. Histogram of signal intensity changes in ROIs on T2 weighted images collected from 24 h after i.v. injection of 3.5 nm, 10 nm, and 20 nm nanoparticles(A). SI indicated signal intensity. The slides of Prussian blue staining show different uptake levels with nanoparticles of different sizes (B).

CONCLUSION

Sub-5 nm UFIO nanoparticles demonstrated higher tumor uptake comparing to IONPs with larger sizes. The transition from T_1 contrast observed in the tumor in the early phase of post-injection to the T_2 contrast at later phase, i.e., 24 hours after the injection of UFIO nanoparticles, may provide a novel capability for one to follow the dynamic process of delivery of UFIO imaging probes to the tumor.

REFERENCES: [1] Larsen EK, Nielsen T, Wittenborn T, et al. *Nanoscale* 2012; [2] Huang et al. 21st ISMRM 2013;3897 [3] Huang et al. 20 th ISMRM 2012;1619.

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