Cellular MRI assessment of magnetic fluorescent bead labeled macrophage accumulation following high intensity focused ultrasound (HIFU) induced damage in a murine model

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Introduction

High intensity focused ultrasound (HIFU) exposures have traditionally been applied in continuous mode in order to generate temperature elevations (≥ 40ºC) for ablating solid tumors. Pulsed (P) HIFU, with lower energy deposition, typically produces non-lethal temperature elevations (≤ 5ºC), enabling subtle mechanical effects (i.e., enlarged gaps between cells) that increase tissue permeability for enhanced drug or gene delivery. Little is known about the potential damaging effects of PHIFU. This study evaluated the effects of HIFU exposures on murine muscle tissue via Cellular MRI (CMRI). Monocytes were labeled in vivo with SPIO/fluorescent nanoparticles prior to application of HIFU at two exposures. CMRI and fluorescent microscopy were used to detect the presence of labeled macrophages in targeted muscle.

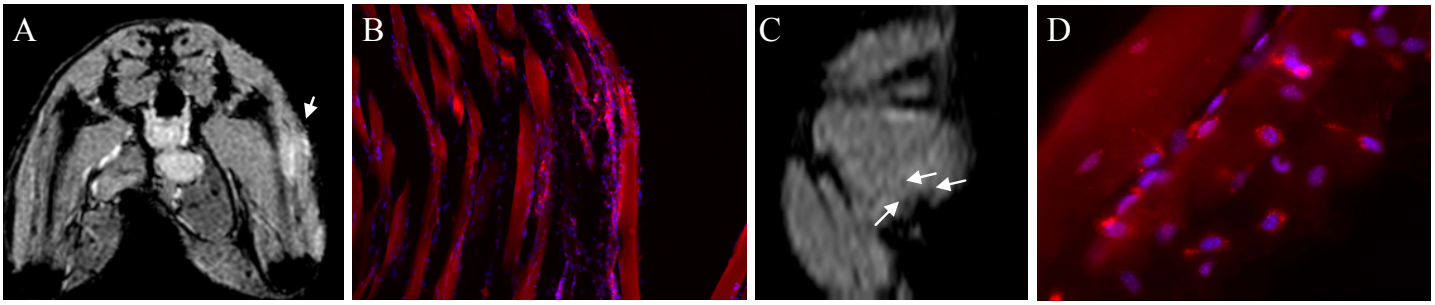
Methods

C3H mice (n=18) were divided into two groups. Group 1 received continuous HIFU to cause ablation at a single 4 sec treatment at 100 W; Group 2 had PHIFU treatments in a 2x3 grid consisting of 100 pulses at a 5% duty cycle that had previously been shown to enable improved delivery of drugs (1). All mice received SPIO/Rhodamine 40 nm particles [8 mg/kg, Biopal Inc] via tail vein 72 hours prior to HIFU. All exposures were targeted in the upper thigh of the animals. On days 1, 2 and 3 post HIFU, three animals from each group were imaged at 3T (Philips Intera) with 4 cm solenoid radiofrequency coil. CMRI was performed with T2W, T2*W and pre-/post- GdDTPA T1W MRI in axial and sagittal planes at 100x100x500 μm resolution. Mice were euthanized and treated/untreated legs were snap frozen for histological confirmation of SPIO labeled macrophages using fluorescent microscopy, prussian blue staining, and immunohistochemistry.

Results

In group 1 mice, T2W and T2*W CMRI showed localized increased signal intensity (SI) in the muscle treated with continuous HIFU. Group 2 mice exhibited smaller areas of high SI regions on T2 and T2*W images. T2*W images from Group 1 and 2 mice showed areas of hypointense regions consistent with susceptibility artifacts in areas around and under HIFU treated regions in dermis and superficial muscle. In Group 1 contrast enhancement was also observed following administration of GdDTPA (data not shown). Fluorescent microscopy of Group 1 mice receiving ablative therapy revealed infiltration of the labeled macrophages compared to rare macrophages found in muscles treated with PHIFU. Figure 1 contains examples T2*W images and fluorescent microscopy from a Group 1 (A,B) and a Group 2 (C,D) mice.

Figure 1



A) T2*W image of HIFU to right leg using ablation exposure. Axial view, Fast Field Echo, TE=15, TR=650, FA=30°. Arrows designate hypointense region characteristic of SPIOs. B) Correlating micrograph of ablation exposure demonstrating numerous DAPI (blue) positive cells with rhodamine beads (red) around muscle (100x). C) T2*W image of HIFU to right leg using PHIFU exposure. Sagittal view, Fast Field Echo TE=15 ms, TR=650 ms, FA=30°. D) Correlating micrograph of PHIFU demonstrating mononuclear cells containing SPIO/rhodamine beads (400x).

Conclusion

In vivo labeling of monocytes with SPIO/fluorescent 40nm beads followed by CMRI and fluorescent microscopy was used to determine the effects of ablative or PHIFU exposures in muscle in a murine model. CMRI demonstrated areas of edema and hypointense regions on T2*weighted images consistent with infiltration of labeled macrophages confirmed by microscopy in animals that had continuous HIFU exposures. Mice treated with PHIFU exposures exhibited smaller regions of edema and hypointense regions confined to superficial muscle and dermis on T2*W images with smaller amount of an immune response within tissues compared to group 1 mice. Being able to evaluate the effects of PHIFU exposures, both spatially and temporally by CMRI, should expedite the development and translation into the clinic for enhancing drug delivery and understanding the pathophysiological response of tissue exposed to HIFU.

Reference: 1) V. Frenkel, *Adv Drug Deliv Rev*, 2008; 60:1193-208.