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$T_{1\rho}$ Mapping for the Evaluation of High Intensity Focused Ultrasound Tumor Treatment

Stefanie J. C. G. Hectors,^{1,2*} Rik P. M. Moonen,^{1,2} Gustav J. Strijkers,^{1,2,3} and Klaas Nicolay^{1,2}

Purpose: This study was aimed to assess the effects of High Intensity Focused Ultrasound (HIFU) thermal ablation on tumor $T_{1\rho}$.

Methods: In vivo $T_{1\rho}$ measurements of murine tumors at various spin-lock amplitudes ($B_1 = 0$ –2000 Hz) were performed before ($n = 13$), directly after ($n = 13$) and 3 days ($n = 7$) after HIFU treatment. T_2 maps were obtained from the measurements at $B_1 = 0$ Hz.

Results: Average tumor $T_{1\rho}$ distributions at the different experimental time points showed a shift toward lower $T_{1\rho}$ values after HIFU for all spin-lock amplitudes, which became larger with increasing spin-lock amplitude at 3 days after treatment. Statistical analysis confirmed a significant effect of spin-lock amplitude on the average change in $T_{1\rho}$ ($\Delta T_{1\rho}$) as compared to baseline at 3 days after HIFU. At 3 days after treatment, $\Delta T_{1\rho}$ values at B_1 above 100 Hz were significantly lower (more negative) than at $B_1 = 0$ Hz (T_2).

Conclusion: Significant changes in tumor $T_{1\rho}$ were observed after HIFU treatment. These $T_{1\rho}$ changes were distinctly more pronounced than HIFU-induced changes in T_2 . The results indicate that $T_{1\rho}$ imaging is sensitive to HIFU-induced tissue changes and may thus be a suitable MR method for the evaluation of HIFU treatment. **Magn Reson Med 73:1593–1601, 2015. © 2014 Wiley Periodicals, Inc.**

Key words: $T_{1\rho}$; spin-lock; high intensity focused ultrasound; cancer treatment response

INTRODUCTION

Magnetic resonance-guided High Intensity Focused Ultrasound (MR-HIFU) is extensively being investigated as a non-invasive technique for thermal treatment of tumors (1). During HIFU ablation, coagulative necrosis is induced in the tissue by rapid heating to a temperature of approximately 60°C (2). Currently, the main clinical application of MR-HIFU is the ablation of benign uterine fibroids (3,4). The last decade, the clinical use of MR-HIFU has been expanded to the treatment of malignant tumors, such as prostate (5,6) and

breast (7) tumors. For the application of MR-HIFU and for the treatment of malignant lesions, it is of key importance that the success of the ablation intervention can be accurately determined and that residual or recurrent tumor tissue can be detected after the procedure. Previously, we have shown by quantitatively comparing MRI data with histology that residual nontreated tumor tissue could be distinguished from successfully treated tumor tissue by multiparametric MRI analysis based on a combination of T_1 , T_2 , and apparent diffusion coefficient data (8). The HIFU-treated and nontreated tumor tissue could not be accurately segmented with any of the individual MRI parameters. Despite the promising results of the above multiparametric MR analysis as a method for HIFU evaluation, other MRI parameters may be more specific to the nature of HIFU-induced changes in the tumor tissue. Tissue destruction by HIFU is mainly based on protein denaturation, which results in cell death by coagulative necrosis. Several advanced MRI methods may report more directly on protein denaturation than T_1 , T_2 , and the apparent diffusion coefficient. Recently, we showed, for example, that amide proton transfer (APT) imaging is a promising MR technique for the evaluation of HIFU treatment. Both directly and 3 days after HIFU treatment distinct regions of decreased APT-weighted signal were observed that corresponded with nonviable tissue observed in histology (9).

Another MRI parameter that is potentially sensitive to HIFU-induced protein denaturation is the longitudinal relaxation time in the rotating frame ($T_{1\rho}$). $T_{1\rho}$ is a measure of the decay of magnetization in the transverse plane during a spin-lock pulse which is applied parallel to the magnetization vector. Tissue $T_{1\rho}$ values are predominantly influenced by protein-water interactions (10). While APT measurements are sensitive to the relatively slowly exchanging amide protons in tissue, on-resonance $T_{1\rho}$ measurements are mainly sensitive to fast exchanging protons. $T_{1\rho}$ imaging may thus provide another yet related source of contrast between HIFU-treated and nontreated tumor tissue. $T_{1\rho}$ imaging has previously been used for multiple applications, including the characterization of cartilage composition and degeneration (11,12), tumors (13–15), stroke (10,16), myocardial infarction (17,18), and neurodegenerative diseases (19). Several preclinical studies have reported on changes in $T_{1\rho}$ after chemotherapy (20) and gene therapy (21–23) of tumors. In these studies, prolonged $T_{1\rho}$ values were observed after treatment. Modeling of the $T_{1\rho}$ dispersion showed that the rise in $T_{1\rho}$ was caused by water accumulation in the tumor tissue and an increase in both the correlation time of water associated with macromolecules and the exchange correlation time (22).

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The goal of the present study was to assess the effects of HIFU treatment on the $T_{1\rho}$ of tumor tissue. To that aim, $T_{1\rho}$ measurements at spin-lock strengths varying between 0 and 2000 Hz were performed before, directly after and at 3 days after HIFU treatment of a murine subcutaneous tumor model. The HIFU treatment consisted of partial ablation of the tumors to allow for internal reference between successfully treated tumor tissue and residual nontreated tumor tissue.

METHODS

Murine Tumor Model

CT26.WT murine colon carcinoma cells (American Type Culture Collection [CRL-2638]) were cultured as a monolayer at 37°C and 5% CO₂ in RPMI-1640 medium (2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, and 1500 mg/L sodium bicarbonate; Invitrogen, Breda, The Netherlands), supplemented with 10% fetal bovine serum (Greiner Bio-One, Alphen a/d Rijn, The Netherlands) and 50 U/mL penicillin/streptomycin (Lonza Bioscience, Basel, Switzerland). Early passages (5–10) of the original American Type Culture Collection batch were used for inoculation.

10–12-week-old male Balb/c mice (Charles River, Maastricht, The Netherlands) were inoculated with 2×10^6 CT26.WT cells subcutaneously in the right hind limb. Approximately 10 days after inoculation, tumors became visible in all animals. The first MRI experiment was performed when tumors had reached a size of approximately 300 mm³. The animal experiments were approved by the animal experiment committee of Maastricht University (The Netherlands).

Study Design

Both HIFU-treated ($n = 13$) and nontreated control ($n = 7$) animals were included in this study. The HIFU-treated animals were subjected to MRI 1 day before ($n = 13$), directly after ($n = 13$) and 3 days after ($n = 7$) HIFU treatment. The time points of the MRI examinations of the control animals corresponded with the MRI examinations of the HIFU-treated animals and are referred to as day -1, day 0, and day 3. The study design comprised three different experimental groups: HIFU-treated animals sacrificed after the MRI examination directly after HIFU ($n = 6$), HIFU-treated animals sacrificed after the MRI examination at 3 days after HIFU ($n = 7$), and nontreated control animals that were sacrificed after the MRI examination at day 3 ($n = 7$).

HIFU Treatment

The HIFU treatment was performed outside the MR scanner with the preclinical Therapy Imaging Probe System (Philips Research, Briarcliff Manor, NY) (24). Treatment was performed under general anesthesia (1–2% isoflurane) and precautionary analgesia (buprenorphine, 0.1 mg/kg s.c., administered 30 min before treatment). The control animals received an equal dose of analgesia at the corresponding time point. A square 4×4 mm² treatment grid consisting of 25 equally spaced treatment points was defined within the tumor area. The treatment

volume typically covered approximately 50% of the total tumor volume to allow for presence of both HIFU-treated and residual nontreated tumor tissue after the HIFU procedure. Treatment settings were: frequency 1.4 MHz, pulse repetition frequency 20 Hz, acoustic power 12 W, duty cycle 50%, treatment time 30 s. A wait time of 120 s was applied between sonication of consecutive treatment points to allow cooling of the tissue to body temperature. During pilot experiments of three mice, a bare-wire thermocouple (80- μ m-diameter T-type thermocouple; T-150A, Physitemp Instruments, Clifton, NJ) was inserted into the tumor tissue to monitor temperature development during HIFU treatment. A temperature of approximately 65°C was reached during sonication with the aforementioned HIFU settings. The thermocouple was not used during HIFU treatment of the experimental groups to prevent non-HIFU-induced damage to the tumor tissue.

MRI Measurements

The MRI examinations were performed with a 7T scanner, equipped with a 72-mm-diameter quadrature radio-frequency (RF) coil (Bruker BioSpin, Ettlingen, Germany). The mice were positioned in a cradle with an anesthesia mask (1–2% isoflurane). Respiration and body temperature were monitored with a balloon sensor and rectal probe, respectively. The body temperature was maintained at approximately 37°C with a warm water pad. The tumor-bearing paw was covered with degassed ultrasound gel (Aquasonic® 100, Parker Laboratories, Fairfield, NJ) for reduction of susceptibility artifacts in the echo planar imaging sequence.

Higher-order local shimming (Bruker MAPSHIM routine) was performed on the tumor-bearing paw to improve B_0 field homogeneity. The $T_{1\rho}$ pulse sequence was performed in two segments and consisted of a spin-lock preparation compensated for B_1 and B_0 field imperfections (25) followed by gradient-echo echo planar imaging read-out (echo time TE = 5 ms, repetition time TR = 2000 ms, and number of averages NA = 2). The acquisition was performed with six different spin-locking times (5, 10, 20, 40, 60, and 80 ms) and eight different spin-lock amplitudes ($B_1 = 0, 100, 250, 500, 750, 1000, 1500$, and 2000 Hz). The acquisitions with the amplitude of 0 Hz can be considered as a T_2 measurement, as the B_0 - and B_1 -compensated spin-lock preparation in the absence of a spin-lock pulse is T_2 -weighted.

In addition to the $T_{1\rho}$ acquisitions, T_2 -weighted imaging (spin-echo, TE = 30 ms, TR = 1000 ms, and NA = 1) and contrast-enhanced T_1 -weighted imaging (spin-echo, TE = 8 ms, TR = 800 ms, NA = 2, acquisitions before and directly after intravenous injection of Dotarem [0.3 mmol Gd/kg]) were performed.

For all MRI acquisitions, a matrix size of 128×128 and a field of view of 4×4 cm² were used. Eight to 12 slices with a slice thickness of 1 mm were defined covering the whole tumor volume.

Data Analysis

Image analysis was performed in Mathematica 8.0 (Wolfram Research, Champaign, IL). $T_{1\rho}$ maps at each spin-

lock amplitude were generated by mono-exponential fitting of the signal intensities at the different spin-lock times. Regions of interest (ROIs) were manually drawn around the tumor tissue on each slice of the T₂-weighted acquisitions. In addition, small ROIs (10–20 pixels) in the muscle tissue surrounding the tumor were drawn on each slice. These muscle tissue ROIs were used to assess the repeatability of the T_{1ρ} measurements.

To analyze the effects of HIFU treatment on the T_{1ρ} in the tumor tissue, histograms of T_{1ρ} in the tumor ROI were generated for each tumor at each time point. The histograms were made for all spin-lock amplitudes. The histogram properties were: 20 bins, bin width 5 ms, and a T_{1ρ} range from 0 to 100 ms. Histograms were averaged for all animals within the different groups (HIFU-treated and control) and spline fitting was performed through these averaged histograms to visualize the T_{1ρ} distributions at the different experimental time points.

From the contrast-enhanced T₁-weighted acquisitions, maps of the signal enhancement after Gadolinium injection were generated according to the following formula:

$$SE = [(SI_{\text{after injection}} - SI_{\text{before injection}}) / SI_{\text{before injection}}] * 100\%,$$

in which SI_{before injection} and SI_{after injection} are the signal intensities in the images before and after contrast agent injection, respectively.

Histological Analysis

Dissected tumors were snap-frozen in isopentane and stored at -80°C . Subsequently, tumors were cut into 5 μm thick sections with a distance of approximately 300 μm between the sections. Multiple sections were collected at each position to allow for different staining procedures. The cryo-sections were briefly air-dried and subsequently stained for nicotinamide adenine dinucleotide (NADH) diaphorase activity to assess cell viability. Sections were incubated at 37°C for 1 h in Gomori-Tris-HCl buffer (pH 7.4) containing β -NAD reduced disodium salt hydrate (Sigma-Aldrich, St. Louis, MO, 0.71 mg/mL buffer solution) and nitro blue tetrazolium (Sigma-Aldrich, 0.29 mg/mL buffer solution). Adjacent cryo-sections were stained with hematoxylin and eosin (H&E) to analyze general morphology. Brightfield microscopy was performed on a selection of the stained sections and consisted of mosaic acquisition of the entire section at $5\times$ magnification.

Statistical Analysis

All data are presented as mean $\pm$ standard deviation (SD). To assess the repeatability of the T_{1ρ} measurements, the effect of time on the average T_{1ρ} values in the muscle ROI in the control animals was tested for significance using an Analysis of Variance method for repeated measures. For both the HIFU-treated and control animals, the average T_{1ρ} values in the tumor ROI at the different time points were statistically compared with a two-sided paired t-test for each spin-lock amplitude. The effect of spin-lock amplitude on the difference in average tumor T_{1ρ} ($\Delta T_{1\rho}$) between before and after HIFU treatment was tested for significance using Analysis of

Variance for repeated measures with the spin-lock amplitude as the within-subject factor. The $\Delta T_{1\rho}$ value was obtained by subtraction of the average T_{1ρ} value in the tumor ROI before HIFU from the average T_{1ρ} value after treatment. In the case the influence of spin-lock amplitude on the $\Delta T_{1\rho}$ proved to be significant, $\Delta T_{1\rho}$ values at the different spin-lock amplitudes were compared separately with a one-sided paired t-test. For all tests, the level of significance was set at $\alpha = 0.05$.

RESULTS

Figure 1a shows representative MRI results as obtained before, directly after and 3 days after HIFU treatment. No clear effects of the HIFU treatment were observed on the T₂-weighted images. This was confirmed by absence of visible changes after HIFU in the T_{1ρ} maps at $B_1 = 0$ Hz, which essentially represent T₂ maps. In contrast, the T_{1ρ} maps at $B_1 = 2000$ Hz showed a pronounced decrease in T_{1ρ} in a large portion of the tumor at 3 days after HIFU treatment. Directly after treatment, only a minor decrease was observed in tumor T_{1ρ} at $B_1 = 2000$ Hz. The signal enhancement maps before HIFU treatment showed an area of decreased perfusion in the tumor core surrounded by regions of high perfusion in the tumor rim. After HIFU treatment, the area of decreased perfusion extended throughout almost the entire tumor tissue. NADH-diaphorase- and H&E-staining on tumor sections obtained at 3 days after HIFU treatment confirmed extensive necrosis in the tumor tissue (Fig. 1b). This necrotic, nonviable region visually corresponded to the areas of decreased T_{1ρ} at $B_1 = 2000$ Hz.

Average tumor T_{1ρ} distributions of the HIFU-treated animals at the different experimental time points are displayed in Figure 2 for all assessed spin-lock amplitudes. T_{1ρ} shifted toward higher T_{1ρ} values at larger spin-lock amplitudes, indicative of presence of tumor T_{1ρ} dispersion in the used range of spin-lock amplitudes. After HIFU treatment, a shift toward lower T_{1ρ} values was observed for all spin-lock amplitudes. At 3 days after HIFU treatment, the tail of the T_{1ρ} distributions toward lower values became larger with increasing spin-lock amplitude, indicative of a larger contrast between non-treated and HIFU-treated tumor tissue at higher spin-lock amplitudes.

Quantitative analysis of the average T_{1ρ} value in the tumors of the HIFU-treated animals at the different experimental time points (Fig. 3a) showed a significant decrease in the average T_{1ρ} at 3 days after HIFU treatment as compared to before treatment for spin-lock amplitudes higher than or equal to 100 Hz. No significant differences between the average T_{1ρ} values before HIFU and directly after HIFU were observed. To further evaluate the contrast between the T_{1ρ} of HIFU-treated and nontreated tumor tissue, average changes in T_{1ρ} values (i.e., $\Delta T_{1\rho}$) were calculated for the entire tumor ROIs (Fig. 3b). No significant effect of spin-lock amplitude on the $\Delta T_{1\rho}$ value between directly after and before HIFU was observed. The average $\Delta T_{1\rho}$ between 3 days after HIFU treatment and before HIFU clearly decreased to larger negative values with increasing spin-lock amplitude. Statistical analysis confirmed a significant effect of

FIG. 1. **a**: Representative example of the MRI results of a tumor-bearing leg before, directly after and 3 days after HIFU treatment. T_2 -weighted images of an axial slice in the tumor-bearing leg are shown in the first column of panels. In the second and third column the $T_{1\rho}$ maps at $B_1 = 0$ Hz, which can be considered as T_2 maps, and at $B_1 = 2000$ Hz are overlaid on the tumor pixels, respectively. In the fourth column, the signal enhancement maps after Gd injection are overlaid on the tumor pixels. The parameter maps were scaled according to the color scale bar which is shown at the right-hand side of the figure. The corresponding parameter range for this scale bar is shown above each column. **b**: NADH-diaphorase (top) and H&E-stained tumor sections (bottom) obtained at 3 days after HIFU treatment at approximately the same location as the MRI slice at 3 days after treatment. A large region of nonviable (NADH-diaphorase negative) and necrotic (H&E) tumor tissue was observed. A small region of residual viable tumor tissue was observed in the left bottom of the section.

FIG. 2. Average $T_{1\rho}$ distributions in the HIFU-treated tumors at the different experimental time points for all assessed spin-lock amplitudes. The lines were generated by spline fitting through averaged histograms.

FIG. 3. **a:** Average $T_{1\rho}$ values in the HIFU-treated tumors at the different experimental time points as a function of the spin-lock amplitude. The error bars represent the SD. * indicates a significant difference between the average $T_{1\rho}$ before and after treatment ($P < 0.05$). **b:** Average $T_{1\rho}$ changes ($\Delta T_{1\rho}$) when comparing both time points after HIFU with before HIFU as function of the spin-lock amplitude. The error bars represent the SD. * indicates a significantly lower (more negative) $\Delta T_{1\rho}$ value than at $B_1 = 0$ Hz ($P < 0.05$). # indicates a significantly lower (more negative) $\Delta T_{1\rho}$ value than at all other spin-lock amplitudes, except 1500 Hz.

spin-lock amplitude on $\Delta T_{1\rho}$ at 3 days after HIFU ($P = 0.048$). The $\Delta T_{1\rho}$ values at B_1 strengths above 100 Hz were significantly lower (more negative) than at $B_1 = 0$ Hz at 3 days after treatment. Furthermore, the $\Delta T_{1\rho}$ value at 2000 Hz was significantly lower than the $\Delta T_{1\rho}$ values at B_1 strengths between 0 and 1000 Hz.

Representative MRI results of the nontreated control animals, which were scanned at three equivalent time points, are shown in Figure 4a. Homogeneous appearance of the tumor tissue was observed on the T_2 -weighted images at all three time points. No visible changes of the $T_{1\rho}$ of the tumor tissue in time were

observed, both at 0 and 2000 Hz. The signal enhancement pattern after Gd injection was similar at all time points and consisted of a less perfused tumor core surrounded by a highly perfused tumor rim. The NADH-diaphorase- and H&E-stained tumor sections, obtained at approximately the same location as the MRI slice at day 3, confirmed general viability of the tumor tissue with small areas of nonviable tumor tissue.

The average tumor $T_{1\rho}$ distributions of the control animals at the different experimental time points are shown in Figure 5. Similar $T_{1\rho}$ distributions were observed for day -1 and day 3 at all spin-lock amplitudes. At day 0,

FIG. 4. **a:** Representative example of MRI results of the tumor-bearing leg of a nontreated control animal at day -1, day 0, and day 3. The layout of the figure is identical to that of Figure 1a. **b:** NADH-diaphorase and H&E-stained tumor sections obtained at day 3 at approximately the same location within the tumor as the MRI slice measured at day 3.

FIG. 5. Average $T_{1\rho}$ distributions in the nontreated control tumors at the different experimental time points for all assessed spin-lock amplitudes. The lines were generated by spline fitting through averaged histograms.

the $T_{1\rho}$ distribution slightly, but not significantly, shifted toward higher $T_{1\rho}$ values as compared to day -1 and day 3.

Figure 6a shows the average $T_{1\rho}$ value in the tumor tissue of the control animals at all time points as a function of the spin-lock amplitude. For all assessed spin-lock

amplitudes, the average $T_{1\rho}$ values did not change significantly in time. In Figure 6b, the average $T_{1\rho}$ value in the muscle tissue is plotted versus the spin-lock amplitude for the control animals at all time points. For each spin-lock amplitude, no significant effect of time on the muscle $T_{1\rho}$ was observed.

FIG. 6. **a**: Average $T_{1\rho}$ values in the nontreated control tumors at the different experimental time points as a function of the spin-lock amplitude. The error bars represent the SD. No significant differences between the average $T_{1\rho}$ values at the different experimental time points were observed for all assessed spin-lock amplitudes. **b**: Average $T_{1\rho}$ values in the muscle tissue surrounding the nontreated control tumors at the different experimental time points as a function of the spin-lock amplitude. The error bars represent the SD. For each spin-lock amplitude, there was no significant effect of time on the $T_{1\rho}$ value of the muscle tissue.

DISCUSSION

In this study, the effect of HIFU treatment on the T_{1ρ} of tumor tissue was assessed. A significant decrease in tumor T_{1ρ} was observed at 3 days after HIFU treatment. The T_{1ρ} decline became significantly larger at higher spin-lock amplitudes. Directly after HIFU treatment, no significant changes of the tumor T_{1ρ} values were observed as compared to before HIFU. As indicated in the Introduction, HIFU-induced tissue damage is mainly based on protein denaturation, which occurs instantly during treatment. The absence of significant effects of the HIFU treatment on the tumor T_{1ρ} immediately after treatment indicates that T_{1ρ} may not be directly sensitive to HIFU-induced protein denaturation.

The significant decrease in tumor T_{1ρ} at 3 days after HIFU treatment was likely caused by latent effects of the HIFU treatment. In contrast to the results presented here, in other preclinical studies on the effect of tumor treatment (gene therapy (21–23) and chemotherapy (20)) increased values of T_{1ρ} were observed after treatment. In the studies on T_{1ρ} after gene therapy, the rise in T_{1ρ} occurred at 4 days after gene therapy, whereas a significant increase in T_{1ρ} was observed already at 18 h after chemotherapy (20). Modeling of the T_{1ρ} dispersion of nontreated tumor tissue and gene-therapy-treated tumor tissue showed that the observed T_{1ρ} rise was caused by an increase of free water and an increase in both the correlation time of water associated with macromolecules and the exchange correlation time (22). The observed decline in tumor T_{1ρ} at 3 days after HIFU treatment reported in the present study was likely caused by opposite effects. HIFU-induced protein denaturation and aggregation could have led to extrusion of water from the tumor tissue because of increased hydrophobic interactions, as has been described in an ex vivo study on thermal treatment of various tissues (26). This extrusion of water could have led to a decrease of the free water proton fraction and thereby have decreased the tumor T_{1ρ} values after HIFU treatment. Furthermore, the correlation time of the exchange between protons of proteins and water molecules could have changed after HIFU treatment. Accumulation of the aforementioned effects probably led to the significant contrast between HIFU-treated and nontreated tumor tissue observed at 3 days after HIFU treatment.

Modeling of the T_{1ρ} dispersion in the tumors before and after HIFU treatment could give additional insights in the effects of HIFU treatment on the macromolecular tissue status. However, accurate fitting of the T_{1ρ} dispersion in the presented data could not be performed, as the maximum spin-lock amplitude (2000 Hz) was too low. In a previous study on T_{1ρ} imaging of gene-therapy-treated gliomas, the T_{1ρ} dispersion could be modeled based on T_{1ρ} data with spin-lock amplitudes up to approximately 6000 Hz (22). In the current study, inclusion of T_{1ρ} values at similarly high spin-lock amplitudes would probably have improved sampling of the T_{1ρ} dispersion. Furthermore, T_{1ρ} measurements at higher spin-lock amplitudes could even further increase the contrast between nontreated and HIFU-treated tumor tissue, as the absolute ΔT_{1ρ} value at 3 days after HIFU was still

increasing at 2000 Hz (Fig. 3b). Unfortunately, T_{1ρ} measurements at higher spin-lock amplitudes were not possible with our current setup, because of hardware restrictions on the maximum coil power. In addition, analysis of the level of protein denaturation in the excised treated tumors could have allowed for further validation of the observed changes in T_{1ρ}. Recently, a method for measurement of the concentration of denatured proteins in ex vivo heart and liver samples that were treated with HIFU-induced histotripsy was described (27). However, the goal of the current study was to initially assess the effects of HIFU treatment on tumor T_{1ρ}. Therefore, ex vivo analysis of the excised tumors was for now restricted to conventional H&E and NADH-diaphorase histology.

Large error bars were observed on the average ΔT_{1ρ} values (Fig. 3b), both directly and at 3 days after HIFU treatment, indicative of a large variation in the HIFU-induced T_{1ρ} change in the different tumors. This was probably caused by the fact that the tumors were treated partially. A fixed treatment area of 4 × 4 mm² was used, which led to a variation in the tumor volume fractions that were ablated. A lower treated tumor fraction leads to presence of a relatively large region of residual, nontreated tumor tissue, causing a smaller change between the tumor T_{1ρ} values before and after treatment. Consequently, the absolute ΔT_{1ρ} values would likely be even larger if the residual, nontreated tumor tissue after HIFU treatment was excluded from this analysis. However, as significant changes between the ΔT_{1ρ} values at the different spin-lock amplitudes were already observed with analysis based on all tumor pixels, analysis with exclusion of nontreated tumor pixels after HIFU was not necessary to demonstrate the enhanced contrast between HIFU-treated and nontreated tumor tissue at higher spin-lock amplitudes.

To investigate the effect of tumor progression on the tumor T_{1ρ} values, nontreated control animals were scanned at the same time points as the HIFU-treated animals. The tumor T_{1ρ} values of the control animals did not significantly change in time, which shows that the observed changes in T_{1ρ} in the HIFU-treated animals could be purely attributed to the effects of HIFU treatment on the tumor tissue. In addition, the average T_{1ρ} value in the muscle tissue surrounding the tumor remained constant for all MRI examinations of the control animals, which is indicative of good repeatability of the T_{1ρ} measurements.

The T_{1ρ} data were qualitatively compared to T₂-weighted imaging and contrast-enhanced T₁-weighted imaging. The effects of HIFU treatment were not visible on the T₂-weighted images, which was supported by absence of visible contrast after HIFU on the T₂ maps (T_{1ρ} at B₁ = 0 Hz). The inadequacy of T₂-weighted imaging for the evaluation of HIFU treatment has been pointed out before in a clinical study of HIFU treatment of prostate tumors (28). The areas of decreased perfusion derived from the contrast-enhanced acquisitions were apparently larger than the areas of decreased T_{1ρ} (Fig. 1). Previously, we have reported a similar finding in a study on APT imaging of HIFU-treated tumor tissue, in which we observed that the areas of decreased contrast agent

uptake after HIFU treatment were significantly larger than the areas of decreased APT-weighted signal. As the areas of decreased APT signals visually corresponded with areas of nonviable tissue in histology, contrast-enhanced T_1 -weighted imaging was found to overestimate the extent of necrosis (9). This overestimation is probably caused by HIFU-induced destruction of tumor-feeding blood vessels (29,30). Vessel destruction leads to diminished perfusion of the tissue that is supplied by the damaged vessel. If this poorly perfused tissue is outside the treatment volume, it will not undergo coagulative necrosis, but may become necrotic in a later stage.

$T_{1\rho}$ measurements have been linked to chemical exchange saturation transfer imaging experiments (31,32). Both methods are sensitive to the chemical exchange between protons of different molecules. Recently, we have shown that APT imaging, which is a variant of chemical exchange saturation transfer imaging, provides significant contrast between HIFU-treated and nontreated tumor tissue (9). APT imaging uses the chemical exchange of protons between amide groups of mobile proteins and water molecules. The on-resonance spin-lock measurements as performed in this study are mainly sensitive to fast exchanging protons for which the exchange rate is much higher than the offset frequency from water, whereas amide protons are in slow exchange with water with an exchange rate of approximately 10–30 Hz (33). The APT and $T_{1\rho}$ measurements are thus sensitive to different proton pools and could, therefore, provide complementary information on changes in the macromolecular characteristics of the tissue. Nevertheless, the spin-lock measurements can be made sensitive to amide protons by using off-resonance spin-lock pulses. Strong similarities between off-resonance spin-lock measurements and chemical exchange saturation transfer imaging of amide proton-containing phantoms have been observed (31).

Clinical translation of $T_{1\rho}$ imaging for the evaluation of HIFU treatment seems feasible. However, the specific absorption rate associated with the relatively long spin-lock pulses limits the maximum spin-lock amplitude. In clinical studies, typically B_1 strengths of 500 Hz are used with spin-lock times up to 60 ms (19,34). In the present study, significant contrast between nontreated and HIFU-treated tumor tissue was already observed at $B_1 = 100$ Hz, which suggests that evaluation of HIFU treatment with $T_{1\rho}$ would be feasible within the clinical specific absorption rate constraints. Nevertheless, the $T_{1\rho}$ contrast after HIFU treatment should be tested in different human tumor types to assess its clinical potential, as the changes in $T_{1\rho}$ after HIFU could be different in other tumors. Furthermore, the dependence of $T_{1\rho}$ on the strength of the B_0 field should be taken into account. $T_{1\rho}$ has shown to decrease with increasing field strength (35). As clinical MR scanners generally have a lower field strength than the 7T preclinical scanner used here, the contrast in $T_{1\rho}$ between treated and nontreated tumor tissue may be influenced by the difference in B_0 field. In addition, the generally lower signal-to-noise ratio and spatial resolution of clinical MR measurements may affect the sensitivity of $T_{1\rho}$ imaging to HIFU-induced tissue changes.

To fully assess the suitability of $T_{1\rho}$ imaging as a biomarker for the evaluation of HIFU treatment, $T_{1\rho}$ imaging should be compared to other MRI parameters in terms of their sensitivity to HIFU-induced changes in the tumor. We have previously shown that APT imaging (9) and multiparametric MRI analysis (8) are both promising methods for the evaluation of HIFU treatment. While a significant change in the tumor APT-weighted signal was already observed directly after HIFU treatment, the multiparametric MR analysis method based on combined T_1 , T_2 , and apparent diffusion coefficient data had the largest sensitivity for HIFU-induced necrosis at 3 days after HIFU treatment. The larger change in $T_{1\rho}$ compared to T_2 at 3 days after HIFU that was observed in the current study suggests that $T_{1\rho}$ is of substantial additive value to the previously described multiparametric analysis. In addition, the $T_{1\rho}$ measurements could be performed at later time points after treatment to assess the effects of tissue repair (36) on tumor $T_{1\rho}$. The 3-days time point in the current study was chosen because it has been reported that the necrotic ablation region is largest in size at that time point after treatment (37).

In conclusion, we have shown that $T_{1\rho}$ mapping gives superior contrast between thermally ablated and nontreated tumor tissue as compared to T_2 mapping. Although only partial tumor ablation was performed, global analysis of the average $T_{1\rho}$ values in the whole tumor already showed a significant difference between tumor $T_{1\rho}$ before and at 3 days after HIFU, indicative of substantial $T_{1\rho}$ contrast between HIFU-treated and nontreated tumor tissue. $T_{1\rho}$ imaging may thus be a suitable MR method for the evaluation of HIFU-induced changes in tumor tissue.

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