MR Guided Pulsed High Intensity Focused Ultrasound Enhancement of Docetaxel Combined with Radiotherapy for Prostate Cancer Treatment

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Introduction

This report summarizes our studies based on the funded research project. The project aims were (1) to determine if focused ultrasound (FUS) increases the cellular uptake of ³H-docetaxel *in vivo*, and (2) to establish whether the increased uptake of docetaxel combining with RT will enhance tumor growth inhibition. The sequence of our studies is as follows. First we performed experiments on an acoustic phantom to determine the optimal ultrasonic parameters. Second, we developed techniques for the treatment of prostate tumor-bearing mice. Third, we verified the enhancement of ³H-docetaxel uptake in prostate tumors with MRgFUS *in vivo*. Finally, we investigated the effect of enhancement of docetaxel (Chemotherapy) by MRgFUS on orthotopically implanted prostate tumor in mice *in vivo*. We summarize the results of these studies in the following sections.

Study on ultrasound output parameters

Pilot experiments were performed on an MRgFUS system with an acoustic phantom provided by InSightec. The purpose of this study was to determine the ultrasound output including frequency, acoustic power and pulse width that are adequate for the enhancement of chemotherapy in mice, without damage to normal tissues. The MR proton resonance frequency shift sequence was used for temperature mapping during the treatment. We assumed that tissue would not be damaged below 42°C. Our results suggested that in order to avoid permanent tissue damage, the acoustic power should be equal or less than 5 W. Our results showed that at 1 MHz and an acoustic power of 4W the 4°C temperature elevation was maintained from 40 joules to 180 joules (sonication time: 10-40 seconds) due to thermal equilibrium. We confirmed that with these ultrasonic parameters there have been no tissue damages (ablation) seen in excised tissues. These phantom measurements provided basic ultrasonic parameters for the MRgFUS system used in our *in vivo* studies (see below). It should be mentioned that 1 MHz was chosen based on the cavitation mechanism, which is consistent with previous studies (i.e. 1, 2).

Tumor models and determination of optimal MR protocols

All animal studies were carried out under the animal protocol (08-19) approved by IACUC (institutional animal care and use committee) at Fox Chase Cancer Center (FCCC). LNCaP (human prostate cancer) cells (10⁶) were injected into the prostates of mice. MRI was performed beginning at 3 weeks after tumor implantation.

In order to use MR guidance for the focused ultrasound treatment, we developed an optimal MR imaging protocol, which allowed us to obtain a higher quality image to visualize the prostate tumor while the scan time remains acceptable for the whole MRgFUS treatment

procedure within approximately 1 h anesthesia time.

A quick scan was performed for initial localization using the fast spin echo (FSE) image sequence at low image quality. The scan time was 0.13 min. It was followed by a high quality image sequence with 7 min scan time in order to identify the tumor target. The MR parameters were: T2-weighted coronal fast spin echo (FSE) sequence; TR/TE=2150/102 ms; Bandwidth: 10.4 kHz; FOV=9 x 9 cm; Matrix: 384 x 384 NEX: 4; slice thickness: 2.0 mm/0.0 sp; frequency direction: SI and the spatial resolution: 0.23 mm. Based on the coronal image a high quality, axial MR images with 4.25 min was obtained. The total scan time was approximately 11 min. Figure 1 show the high quality image that was used for our MRgFUS treatment planning.

Figure 1. MR images showing the prostate tumor in the coronal view

Experimental setup

The mice were treated under general anesthesia. A gel pad was placed on the treatment table in line with the transducer. Degassed water was used for the interface between the treatment table and the gel pad for the acoustic coupling. Care was taken to eliminate any air bubbles between the interfaces. The gel pad was warmed to approximately 37 °C and a shallow hole measured about 2cm x 3cm with 8mm in depth was made. The hole was located in the center on the top of the gel pad and filled with warm degassed water. The animal was carefully placed on the gel pad. The animal stomach was in line with the hole in contract with the degassed water in a prone position. A 3-inch surface coil was placed around the animal to receive the MR signals. A small acoustic phantom was placed beside the animal for the purpose of the ultrasonic beam verification. A plastic glove filled with warm water was placed on the animal to maintain its body temperature. Three localization MR sequences were performed to ensure there were no gas bubbles in the interface between the gel pad and the animal skin (figure 2).

Figure 2. The animal set up for MRgFUS treatment for this project

³H-Docetaxel uptake in prostate tumors treated with MRgFUS *in vivo*

This study was carried out under the protocols approved by the radiation safety committee and IACUC at FCCC. When prostate tumors reached the size of $160 \pm 10 \text{ mm}^3$ on MRI, FUS treatment was preformed using an ExAblate 2000 (InSightec system) with a 1.5 T GE MR scanner. The animals were randomly divided into 3 groups (n=8 per group). The 3 groups are: Group 1, HIFU treatment + ³H-docetaxel; Group 2, FUS treatment only and Group 3, as control. For group 1, each mouse was treated under general anesthesia with pulsed FUS using 1 MHz; 4 W of acoustic power and the 81 mode setting (5 Hz frequency with 0.1s power on, 0.1s power off) for 60 seconds under MR guidance. Immediately after FUS treatment animal

received a single dose of i.v injection of docetaxel at 15 mg/kg mixed with ^3H -docetaxel at 50 uCi/kg in 150 μl . The dose determination was based on the published article (3). Animals in group 2 were treated the same as for group one but without FUS treatment. Animals were sacrificed 30 min after i.v injections and tumors were removed and processed. The radioactivity of ^3H -docetaxel in the tumor tissue was quantitatively measured by a liquid scintillation counter.

Our study results showed that all animals tolerated the MRgFUS treatment well. There were no treatment-related adverse events including skin toxicity. Our data show increased ^3H -docetaxel concentration in tumor in the MRgFUS treated group (1079 ± 132 cpm/75 μg) vs. those without MRgFUS treatment (524 ± 201 cpm/75 μg) with $P = 0.037$ (figure 3).

Figure 3. Comparison of ^3H -Docetaxel between with FUS treatment and without FUS treatment group (n=8).

Effect of Docetaxel with MRgFUS on prostate tumor growth *in vivo*

The purpose of this study was to investigate whether or not docetaxel + FUS will improve the prostate tumor control. When tumors reached the volume of 47 ± 3 mm^3 on MRI, treatment was performed. The animals were randomly divided into 3 groups (n=5/group). For group 1, mice were treated with MRgFUS once a week for two consecutive weeks. Animals were treated with pulsed ultrasound using 1 MHz; 5 W acoustic power and the 81 mode setting (5 Hz frequency with 0.1s power on, 0.1s power off) for 60 seconds for one sonication. Multiple sonications (4-6) were used to cover the whole tumor volume. Immediately after the MRgFUS treatment, docetaxel (10mg/kg) was injected by tail vein. The animal was allowed to survive for 4 weeks after treatment. The tumor growth was monitored on MRI (7 T). Animals in Group 2 were treated with docetaxel injection only. Animals in group 3 were treated with FUS only and group 4 was used as control.

Table 1. Comparison of tumor volumes (TV) between the groups (n=5). TX=treatment

Groups	TV (mm^3) before Tx	TV (mm^3) 1 wk after Tx	TV (mm^3) 4 wks after Tx	Relative TV before Tx	Relative TV 1 wk after Tx	Relative TV 4 wks after Tx
FUS+Docetaxel	41.4 ± 4.0	32.6 ± 3.2	30.9 ± 4.60	1.0 ± 0.11	0.79 ± 0.05	0.77 ± 0.1
Docetaxel	43.9 ± 2.8	39.8 ± 9.9	65.0 ± 23.9	1.0 ± 0.06	0.90 ± 0.21	1.52 ± 0.6
FUS	48.0 ± 6.2	96.9 ± 10.3	177.6 ± 34.4	1.0 ± 0.13	2.15 ± 0.5	4.29 ± 1.3
Control	50.0 ± 3.9	133.0 ± 17.5	328.5 ± 54.2	1.0 ± 0.06	2.69 ± 0.48	6.77 ± 1.3

Our results showed that all animals in groups 3 tolerated FUS well. As expected animals in group 1 and 2 showed weight loss (approximately 15-20% of the body weight) during chemotherapy and gradually the weight recovered after the treatment. There has been no animal death associated with the treatment. Table 1 summarizes tumor growth before and after treatment for each group. The data was further illustrated in Figure 4. The relative tumor volume in MRgFUS + docetaxel treated group was 0.79 ± 0.05 1 week after and 0.77

± 0.1 4 weeks after treatment, respectively, while for the group that received only docetaxel it was 0.90 ± 0.21 and 1.52 ± 0.6 at 1 week and 4 weeks, respectively. Both groups showed smaller tumor volumes compared to the control group. An abstract on the results of this study has been submitted to the AAPM annual meeting (July 2010).

It is interesting to see that the tumor is smaller in the FUS group compared with the control group (4.3 ± 1.3 vs. 6.8 ± 1.3) 4 weeks after treatment. Further studies are warranted to investigate the effect of non-thermal FUS on tumor growth control.

Figure 4. Comparison of relative tumor volume before and after the treatment for each group (n=5)

Discussions

To study the effect of docetaxel with MRgFUS on prostate tumor in mice *in vivo* experiments were initially performed using i.p injections with docetaxel. The reason to use i.p injection was based on an assumption that it might not be possible for multiple chemo-drug injections by tail vein in mice although we believed that the i.v injection would be more effective. However, after i.p injections for three consecutive weeks 40-50 % of the animals suffered from severe local GI toxicity showing expanded intestines due to compromised intestinal peristalsis in the docetaxel treated groups. In addition, 75 % of the animals in the group of FUS+docetaxel (i.p) +RT could not tolerate the entire treatment. After we modified our treatment protocol to deliver treatments in 2 consecutive weeks instead of 3 consecutive weeks there were still 17-33 % of animals that could not tolerate the i.p injection. Finally we modified our protocol again to treat with multiple i.v injections and our results showed that animals in the docetaxel groups treated with i.v injections tolerated the treatment well.

Conclusions: Our preliminary results show that MRgFUS has the potential to increase the efficacy of docetaxel in inhibiting prostate cancer growth.

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