1J-1  1:30 p.m.

A 500 ELEMENT ULTRASOUND PHASED ARRAY SYSTEM FOR NONINVASIVE FOCAL SURGERY OF THE BRAIN- A PRELIMINARY RABBIT STUDY WITH EX VIVO HUMAN SKULLS

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Purpose: To test a prototype Magnetic Resonance Imaging (MRI) compatible focused ultrasound phased array system for trans-skull brain tissue ablation using ex vivo human skulls and an animal model. Methods: Rabbit thigh muscle (N=3) and brain (N=10) were sonicated with a prototype, hemispherical 500-element ultrasound phased array operating at frequencies between 700 and 800 kHz. An ex vivo human skull sample was placed between the array and the animal tissue. The temperature elevation during 20-30 s sonications were monitored using MRI thermometry. The induced focal lesions were observed in T2 and contrast-enhanced T1-weighted Fast Spin Echo images. Whole brain histology evaluation was performed after the sonications. Results: Sharp temperature elevations were produced both in the thigh muscle and in the brain. The temperature elevations per applied acoustic watt during 20 s sonications in the thigh muscle and brain were 0.04 +/- 0.03C and 0.022 +/- 0.010C, respectively. The high power sonications (600 - 1080 W) produced peak temperature up to 55C and focal lesions that were consistent with thermal tissue damage. The lesion size was found to increase with increasing peak temperature Conclusions: This study demonstrates that it is possible to create ultrasound-induced lesions in vivo through a human skull under MRI guidance with this large-scale phased array device.

1J-2  1:45 p.m.

FOCUSED ULTRASOUND TRANSDUCTION OF MOLECULAR DEATH SIGNALS

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Our knowledge about the transduction of mechanical stress on cell signaling and cellular response is limited. Here we use focused ultrasound (FUS) as a versatile tool to locally transduce mechanical stress into specific molecular death signaling (apoptosis) and cellular response in three human cancer cells (Tk6 lymphoblasts) that differ in their p53 status. Localized apoptosis induction is interesting, because modulating the apoptosis cell death machinery is an attractive and obvious therapeutic cancer strategy. FUS (calibrated by using a membrane hydrophone with a spot size of 0.5 mm; 0.68 MHz frequency, range 0 to 15 MPa pressure amplitude, 10 ms pulse length, 1 Hz pulse frequency, 60 s total exposure time) was delivered vertically to cells plated in a 12-well dish from the bottom. To each well either Albunex human albumin microspheres (107 bubbles/ml) to increase cavitation activity was added, or an equal volume of phosphate-buffered saline (PBS). A sterile membrane was placed over the plate, and an extension of the outer walls was filled with water at 37°C to avoid air bubbles, reflection, and scattering. We found that exposure to FUS (peak pressure 1.5 MPa) induced localized apoptosis and tumor cell death. In DNA chip analysis augmented with Western, FACS, and ELISA, we detected that FUS treatment upregulated cellular signaling cascades involved in apoptosis (p53, p21, Thy1 (CD 90)) and oxidative stress response (ferritin), while bcl-2, ribosomal proteins and superoxide dismutase (SOD) were downregulated. Cell proliferation inhibition, apoptosis induction and G1 arrest correlated with p53+ status, but were also present in p53- cells that primarily exhibited G2 arrest. Free radical quantification using ESR spectroscopy suggests that both direct ultrasonic mechanical effects and indirect sonochemical effects via cavitation-induced reactive oxygen species play a causative role. Clinically, these results indicate, that FUS in a specific “dose window” may become a new noninvasive strategy in cancer therapy below the well known doses that primarily induce heat. Furthermore, we show that FUS is a powerful in vivo tool that can be used to discover how specific pathways are triggered by mechanical signals.

ULTRASOUND RADIATION FORCE ON ACOUSTICALLY-ACTIVE LIPOSHERES FOR DRUG DELIVERY

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In this study, we first seek to quantify the effects of radiation force on acoustically active lipospheres (AALs). These agents are created by ImRx Therapeutics, Inc and are similar to typical ultrasound contrast agents in structure and material components, except they include a triacetin oil layer in the fluid shell. This oil layer can be used as a solubilizing vehicle for a variety of hydrophobic drugs. Single AAL experiments revealed that the AALs are displaced a distance on the
same order of magnitude as typical contrast agents when exposed to ultrasound pulses. On average the AALs were displaced by 1.24 nm per cycle, while the typical contrast agent was displaced by 2.96 nm per cycle at a frequency of 2.5 MHz and a pressure amplitude of 225 kPa. This displacement is frequency dependent with the largest displacement magnitude occurring near the resonance frequency. In the microvasculature, blood velocity ranges from 1 to 10 mm/s, and therefore a transducer with a 1 mm focus can insonify an agent for 0.1 to 1 seconds in a capillary. Thus, for the above parameters, the agent could be exposed up to 1 million cycles at a frequency of 1 MHz, producing a displacement of up to 1.96 mm. We will present the results of in vitro studies that include human melanoma cell (A2085) and endothelial cell monolayers on Thermonox plates and flowing in mock vessels. The AALs with either Sudan Black or 5-dodecanoylaminofluorescein incorporated within the oil layer are exposed to ultrasound in the cell chambers. Transfer of the dye to cells is demonstrated for sufficient ultrasound parameters. Dye transfer is quantified as a function of the initial concentration and ultrasound parameters, and the resulting dye and fluorescence magnitudes will be presented. A drug delivery scheme based on these vesicles could locally deliver chemotherapy to tumors, thereby significantly decreasing toxic side effects.

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1J-4  2:15 p.m.

IN VIVO COMPARISON OF MULTIPLE PULSE AND CW STRATEGIES FOR MICROBUBBLE-ENHANCED ULTRASOUND THERAPY

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Previously, we showed that microbubble introduction effectively reduces the amount of propagated energy required to produce tissue damage in vivo; we determined threshold exposures for tissue damage and also microbubble concentrations useful for ultrasound therapy. In the current study, our objectives are: 1) to determine, both without and with microbubbles, if repeated, short duration, high amplitude ultrasound exposures (multiple pulses, MP) produce greater tissue damage than single, CW exposures of the same amplitude and total energy; 2) to determine if there exists an “ideal” pulse duration (PD) and/or pulse repetition frequency (PRF) for ultrasound therapy with MP; and 3) to determine if the previously observed efficacy of microbubbles is applicable to MP therapy. In 10 canines, kidneys were externalized and insonified by a highly focused, 1.44 MHz therapy transducer. One kidney was insonified with no microbubbles present while the opposite kidney was insonified during 1 µL/kg/min continuous Optison® infusion. All exposures had \( p_r = 8.2 \) kPa and \( \mathcal{I}_{SPPA} = 3200 \) W/cm². CW exposures ranged from 100 µs to 5 s. MP exposures had PD=5 & 10 µs; PRF=1, 2.5, 5, 10, 15, 20, 25, & 50 kHz; and N pulses equaling the CW duration divided by PD (Because of ringing, each MP exposure has slightly less energy than the corresponding CW exposure). The
result of each exposure was evaluated for 6 distinct visible characteristics ranging from "no damage" to "mechanical disruption". Complete tissue damage quantification by histological evaluation is in progress. Thus far, we've made the following observations: 1) both with and without microbubbles, damage produced by MP was similar or greater in size and displayed less thermal coagulation than damage produced by CW; 2) for PD=5 & 10 μs, the amount of tissue damage remained fairly constant for PRF above 10kHz with microbubbles and 10-25 kHz without microbubbles; and 3) approximately 10x fewer pulses were required to produce similar amounts of damage with microbubbles than without microbubbles. These results indicate that it may be possible to achieve greater tissue damage in ultrasound therapy with less propagated energy by using a combination of microbubbles and multiple pulse sequences.

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KIDNEY BLOOD FLOW OCCLUSION BY ACOUSTIC DROPLET VAPORIZATION

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Acoustic Droplet Vaporization (ADV) has been introduced with the potential application of tumor treatment via occlusion and subsequent necrotic ischemia in the targeted region. Previously it has been shown that blood flow reductions of up to 34% could be realized by B-mode ADV in localized target tissue. In this study an entire organ was target for occlusion. Lepus kidneys were targeted for bubble production and subsequent vascular occlusion of the organ. The animal’s physiological response was monitored before, during, and after ADV.

New Zealand white rabbits were anesthetized by isoflurane and their left kidney was externalized. A 12 cm diameter water tank was placed over the kidney and an imaging array and single element transducer were positioned in the tank with direct access to the kidney’s vasculature and renal artery, respectively. Filtered droplet emulsions (diameter <5 μm) were injected intravenously (IV) or intra-arterially (IA) into the left heart during insonification of the renal artery and the extent of blood flow reduction by ADV was compared to the untreated right side kidney. Blood flow before and after each treatment was measured using fluorescently stained PVC spheres (diameter 15 μm) injected IA into the heart’s left ventricle. Flow cytometry analysis of kidney tissue samples and reference blood from the femoral artery allowed the estimation of regional blood flow (mL g⁻¹ min⁻¹) inside the tissue samples. Physiological data such as blood oxygenation and heart rate were monitored via a pulse oxymeter.

Bubble production was possible for IV as well as IA administered emulsions. A maximum regional blood flow reduction of >95% (90% for total organ) was found for occlusion based on bubbles from ADV of IA injected droplet emulsions. After treatment of the left kidney, the control kidney on the contralateral side showed an increase in regional blood flow of up to 30% relative to the pre
ADV baseline. Hyper echogenicity from ADV of IA injections was monitored for approximately one hour. This is enough time for the onset of cell death and subsequent tumor treatment via ischemic necrosis. There was no systematic change in the animal’s heart rate and oxygenation after IA injection of filtered droplets.

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1J-6 2:45 p.m.

DURATION OF ULTRASOUND AND BUBBLES ENHANCED CELL MEMBRANE PERMEABILITY
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Purpose: Ultrasound (US) has shown the ability to modulate the cell membrane permeability in a process known as sonoporation. In addition, the sonoporation process has been proven to be amplified when US is associated with contrast microbubbles. The purpose of this study is to quantify the duration of the sonoporation process for external molecules with different sizes. Method: monolayers of Chinese Hamster Ovary (CHO) cells, fixed on a membrane, were used and 3 fluorescent-labeled dextran molecules (10, 40 and 70 KDa) were used as markers. The US settings consisted of a burst of 10 cycles with a frequency of 1 MHz at acoustic pressures between 0.2-1.0 MPa with a pulse repetition rate of 20 Hz. CHO cells were irradiated at 37°C for 2 minutes after addition of an experimental contrast agent BR14 (Bracco Research, Geneva, Switzerland) in a ratio of 1 bubble:1 cell. The cells were incubated with the 3 markers at t=0 sec, 10 sec, 30 sec, and 60 sec after US was turned off. The respective uptake levels were measured 10 minutes after the addition of the markers. Results: the control cells (no US) showed no molecular uptake. Maximum uptake was reached when the cells were incubated with the markers immediately after the US exposure (t=0). The uptake decays with time for all the markers and reaches only 20% when the incubation was performed 60 sec after US exposure with the largest marker dye. The decay rate depends on the marker size but not on the MI. The relationship between decay rate and incubation time is nonlinear where a quick decrease in uptake was observed for the largest dye. Conclusions: A negative correlation between maximum uptake and time after the turn off US is demonstrated. Moreover, a higher maximum uptake level at the moment of ultrasound turn off results in a faster decay in uptake. In conclusion the duration of enhanced membrane permeability is limited with a maximal existence of less than 60 sec. This depends on the size of the molecules but not on MI.

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