4pBA3. From high-frequency to low-frequency cell death detection: quantitative ultrasound evaluation of tumor response in breast cancer


*Corresponding author’s address: Imaging Research | Radiation Oncology | Medical Biophysics, Sunnybrook Health Sciences Centre | University of Toronto, Toronto, M4N 3M5, ON, Canada, Gregory.Czarnota@sunnybrook.ca

Pre-clinical and clinical studies were undertaken investigating the efficacy of ultrasound to quantify cell death in tumor responses with cancer treatment. Animals bearing tumours (n=48), and patients (n=24) with locally advanced breast cancer received various therapies including for patients anthracyline and taxane-based chemotherapy treatments over four to six months. Tumour cell-death was assessed in specimens after treatment using histopathology. Pre-clinical ultrasound data collection was carried out at low-frequency and high-frequency. For human imaging, low-frequency ultrasound data were collected 5 times during neoadjuvant chemotherapy. Data indicated considerable increases in ultrasound backscatter in animal tumours after treatment. Similar findings were observed in patients who clinically responded to treatment. Patients assessed as responding poorly demonstrated significantly lower increases. Increases in 0-MHz intercept followed similar trends while increases in spectral slope were observed locally from tumor regions demonstrating increases in tissue echogenicity. This study demonstrates the potential of ultrasound to quantify changes in tumours in response to cancer treatment administration in a pre-clinical and clinical setting. The results indicate that such responses can be detected early during a course of chemotherapy in patients and should permit ineffective treatments to be changed to more efficacious ones potentially leading to improved treatment outcomes.

Published by the Acoustical Society of America through the American Institute of Physics
BACKGROUND

High-frequency ultrasound has been used previously to detect cell death. Specifically, to date, high-frequency ultrasound has been used to detect apoptotic cell death resulting from chemotherapy, photodynamic, X-ray radiation and ultrasonically activated anti-vascular micro-bubble therapies in a variety of in vivo mouse model (Banihashemi et al., 2006; Vlad et al., 2008, 2009). Those studies have demonstrated up to 16-fold maximal increases in observed backscatter signal intensity accompanied by increases in spectral parameters such as spectral slope, 0-MHz intercept, and mid-band fit (MBF) quantitative parameters which can be related to effective scatterer size, acoustic concentration, and both, respectively (Lizzi et al., 1987, 1997). Similar methods have been used in a variety of other ultrasound tissue characterization applications, such as in the diagnoses of prostate cancer, liver and cardiac abnormalities and the differentiation of benign fibroadenomas from mammary carcinomas and sarcomas (Feleppa et al., 1996; Guimond et al., 2007; Oelze et al., 2004; Yang et al., 2007).

High-frequency 20-50 MHz ultrasound has an increased imaging resolution (30-80 μm) when compared to clinically employed conventional-frequencies of 1 to 20 MHz (80 μm - 1.5 mm). However, it suffers from a limited tissue penetration depth, restricting its use to superficial tissue sites near the skin surface. Whereas the application of conventional-frequency ultrasound carries a decreased imaging resolution, it benefits from a substantive tissue penetration capacity. Such an advantage potentially allows for non-invasively monitoring the effects of therapy on deeper malignant tissues such as breast, kidney and liver cancerous tumours. This has motivated a number of recent studies into the capacity of conventional-frequency ultrasound in the detection of cancer treatment effects, effectively laying the groundwork for clinical evaluations at lower ultrasound frequencies (Azrif et al., 2007; Falou et al., 2012; Sadeghi-Naini et al., 2012).

MATERIALS AND METHODS

We carried out comparisons of high versus low-frequency ultrasound in preclinical tumour models. Experiments were conducted using xenografted human prostate (PC3) and breast (MDA-MB-231) tumours (7-9 mm size) on hind leg of severe combined immunodeficiency disease (SCID) mouse, 4-5 weeks after injection of cells subcutaneously. Experimentation used 32 PC3 and 16 MDA bearing mice; each tumour type was divided equally into four treatment categories, one of which remained untreated as a control group.

For the PC3 tumour model, the first treatment group received a 8 Gy radiation using 160 kVp X-rays at 200 cGy/minute (Faxitron Corporation, Illinois, USA). The second group was administered vascular-targeting therapy consisting of a high-concentration dose (3% v/v) of ultrasonically-stimulated microbubbles (Definity, Bristol-Myers Squibb, New York, USA) to induce vascular disruption. The last group received a combination therapy consisting of the anti-vascular ultrasound and bubble therapy followed immediately by the X-ray radiation as above. Microbubble stimulation in these groups was carried out using an in-house constructed system with an ultrasound excitation pulse centered at 500 kHz and a matched unfocussed transducer (Valpey Fisher, Hopkinton, MA, USA) with a pulse repetition frequency of 3 kHz and peak negative pressure of 570 kPa for a total administration time of 750 ms over a 5 minute period in order to avoid any potential hyperthermic effects on tissue. This microbubble treatment alone causes vascular destruction leading to apoptosis and when combined with radiation yields rapid apoptotic cell-death within the central core of these human xenograft tumours and is described elsewhere.

The MDA tumour model was treated with Paclitaxel-Doxorubicin (150 mg/m², 50 mg/m²) through intravenous tail injection, to mimic human chemotherapy administration. Each MDA treatment group received a different period of treatment, i.e. 0, 4, 12, and 24 hours, respectively. The purpose of the variation of treatment-length was to determine its effects on the tumour response and the sensitivity of quantitative ultrasound to detect it.

Ultrasound data was collected from PC3 tumours prior to and 24 hours following treatment, a time found to be optimal and potentially clinically relevant for response detection. MDA tumours were imaged prior and immediately after their period of chemotherapy. Conventional-frequency ultrasound and RF data were acquired with a Sonix RP, (Ultrasoundx Vancouver, Canada) system utilizing an L14-5/38 transducer pulsed at 10 MHz with a central frequency of ~7 MHz, focused at 1 cm depth, and sampled at 40 MHz. High-frequency data was acquired with a Vevo 770 system (Visual Sonics, Toronto, Canada) using a transducer (RMV-710B) pulsed at 25 MHz with a central frequency of ~20MHz, focused at 9 mm depth, and sampled at 420 MHz. Both systems were used to collect three dimensional data with scan plane separations of 0.5 mm in the conventional-frequency data and 100 microns in the high-frequency data. Immediately following imaging where indicated, animals were killed and tumours excised for histopathological examination.
To investigate the use of this low-frequency methodology in human patients a clinical study was undertaken investigating the efficacy of ultrasound to quantify cell death in tumor responses with cancer treatment. Patients (n=24) with locally advanced breast cancer received anthracyline and taxane-based chemotherapy treatments over four to six months. The majority of patients went on to have a modified radical mastectomy and correlative whole mount histopathology. Data collection was carried out using an Ultrasonix-RP and an L15-5 6cm transducer pulsed at 10 MHz with RF data collected 5 times during neoadjuvant chemotherapy.

RESULTS/DISCUSSION

Ultrasound-based biomarkers related to acoustic backscatter power (mid-band fit), effective scatterer size (spectral slope) and concentration (0-MHz intercept) were determined for both tumour types before and after treatment. For both PC3 tumours treated with ultrasound-stimulated microbubbles in order to enhance radiation effects and for MDA tumours treated with chemotherapy, in responding tumours, the presence of cell-death was reflected in changes in ultrasound spectra for the treated tumours. Both treated tumour models exhibited cell-death in gross morphological staining by TUNEL and ISEL.

Data from chemotherapy patients indicated increases of approximately 9 dBr (+/-1.67) maximally in ultrasound backscatter in patients who clinically responded to treatment. Patients assessed as responding poorly demonstrated significantly lower increases (2.3 +/- 1.7 dBr). Increases in 0-MHz intercept followed similar trends while increases in spectral slope were observed locally from tumor regions demonstrating increases in tissue echogenicity.

These studies demonstrate the potential of high-frequency and low-frequency ultrasound to quantify changes in tumours in response to cancer treatment administration in pre-clinical and clinical settings. The results indicate that such responses can be detected early during a course of chemotherapy or other treatments and should permit ineffective treatments to be changed to more efficacious ones potentially leading to improved treatment outcomes.

REFERENCES