Ultrasound in Med. & Biol., Vol. 38, No. 7, pp. 1212–1221, 2012

0301-5629/$ - see front matter

ELSEVIER

doi:10.1016/j.ultrasmedbio.2012.01.028

● *Original Contribution*

QUANTITATIVE ULTRASOUND FOR THE MONITORING OF NOVEL MICROBUBBLE AND ULTRASOUND RADIOSENSITIZATION

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(*Received* 8 *September* 2011; *revised* 23 *January* 2012; *in final form* 27 *January* 2012)

Abstract—There is a need for cancer imaging to provide "real-time" information about the metabolic and cellular responses of tumours. Quantitative ultrasound techniques have recently been demonstrated to be a potential method of assessing tumour response at the cellular level. Anti-cancer treatments administered to xenograft-bearing mice consisted of radiotherapy and a novel antivascular therapy utilizing encapsulated microbubble agents in the presence of ultrasound. Radiation dose and microbubble concentrations were varied and the treatment modalities were given in combination to assess the possible enhancement of tumour cell death. Quantitative methods were used to non-invasively assess responses. Results demonstrated statistically significant changes in backscatter parameters (midband fit, spectral intercept) in tumours treated with high doses of radiotherapy or a high concentration of microbubbles. Combined treatments demonstrated further increases in ultrasound parameters. Histopathologic assessment was used and tumour cell death was found to correlate with increases in ultrasound parameters. (E-mail: Gregory.Czarnota@sunnybrook.ca) **© 2012 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Quantitative ultrasound, Cell death, Microbubble, Antivascular therapy, Radiation.**

INTRODUCTION

Tumour response imaging

The accurate and predictive medical imaging of tumour response during the course of treatment has the potential to shift the paradigm in oncology from predetermined treatment regimens toward individualized and adaptive treatment pathways based on tumour response "biomarkers" and early assessments of tumour response (reviewed in Brindle 2008). With an increasing number of cancer therapeutics available including targeted antibodies, antivascular agents, chemotherapy and radiation, there is an increasing need for more personalized treatments to optimize efficacy while avoiding excessive and unnecessary toxicity. It may be possible, given recent advances in methods, to use new quantitative ultrasound imaging techniques to personalize therapy.

Common imaging modalities used to image tumour response currently include X-rays, computed tomography and magnetic resonance imaging. Tumour size and volume are measured but may take weeks to months after the commencement of treatment to demonstrate response. Positron emission tomography and magnetic resonance spectroscopy can often be used to demonstrate metabolic responses at an earlier time (Brindle 2008) but remain costly at this time and are not widely available. Ultrasound represents an alternative noninvasive, inexpensive, imaging modality that has the potential to detect early tumour response linked to changes occurring at the cellular level during the course of cancer treatment.

High frequency ultrasound can be used to detect tumour cell changes associated with cell death, such as DNA condensation and fragmentation caused by cancer treatments (Czarnota et al. 1999). Scattering properties of the tumour including acoustic scatterer size, acoustic concentration, impedance and spatial organization can be affected by anti-cancer treatments and subsequently result in increases in ultrasound image intensity (Hunt et al. 2002; Kolios et al. 2002; Taggart et al. 2007). These changes can be quantified through the analysis of radio-frequency (RF) spectral data that is received by all ultrasound devices prior to translation to a clinical

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image. These principles have been demonstrated in multiple tumour types and varying therapeutic modalities (reviewed in Czarnota and Kolios 2010).

For example, early work *in vitro* by Czarnota et al. (1997, 1999) utilized highly controlled models of acute myelogenous leukemia (AML) cells treated with cisplatinum to induce apoptosis and separately colchicine and enzymes to study the effects of nuclear condensation. These experiments established that high frequency ultrasound backscatter can increase by nearly 12 dBr (decibels, normalized to a standard quartz reference) with apoptotic cell death and nuclear condensation. In addition, an increase in the spectral slope parameter was associated with a decrease in nuclear size, which corresponded closely with theoretically predicted values (Kolios et al. 2002). Vlad et al. (2005) examined *ex vivo* liver specimens with ultrasound to assess ischemic changes causing cell death and found backscatter increases in the range of 4–9 dBr. Another *ex vivo* study reported by Oelze et al. (2004) examined mammary tumours with 8.5 MHz ultrasound immediately after euthanizing the animals. In that study, RF analysis was used to differentiate benign mammary fibroadenomas from breast cancer xenografts based on the scatterer size and acoustic concentration of specimens. Similar ultrasound tissue characterization techniques have been used to evaluate and differentiate structures within the eye, prostate gland and kidney (Lizzi et al. 1997, 2006).

Noninvasive ultrasound-based tumour response imaging *in vivo* is a relatively recent development. The first preclinical application of quantitative ultrasound to monitor tumour response was reported by Banihashemi et al. (2008) who evaluated malignant melanoma tumours in mice prior to and after photodynamic therapy. Ultrasound backscatter parameters correlated with cell death; peak ultrasound intensity was observed 24 hours after treatment when maximal cell damage was evident. A similar *in vivo* study by Vlad et al. (2009) studied head and neck carcinoma xenografts before and after radiotherapy treatments. The tumours exhibited an increase of integrated ultrasound backscatter of up to 8.2 dB 24 h after radiotherapy and large hyperechoic regions corresponding to histologic areas of cell death were seen; the spectral slope parameter also increased after radiotherapy. Here, for the first time, we use high frequency ultrasound in a similar manner, to monitor *in vivo* tumour responses to novel vascular disrupting combined radiotherapy and microbubble-based treatments.

Ultrasound and microbubble enhancement of radiation treatment

Radiation therapy causes tumour cell death through a complex process of free radical production and DNA strand breaks, resulting in mitotic catastrophe and other forms of cell death. Current radiobiology research also suggests that alterations of the tumour stroma, microenvironment and microvasculature are closely linked to radiation response. Garcia-Barros et al. (2003) demonstrated that for high single-fraction doses of radiotherapy, endothelial cell death and ceramide pathway activation was a requisite for tumour cell death, whereas with low doses, this was inhibited by hypoxia-inducible factor. In addition, antivascular and anti-VEGF agents are being evaluated presently by others in conjunction with radiation in an attempt to exploit this dependence on the microvasculature (Nieder et al. 2006).

Microbubbles have been used successfully as diagnostic ultrasound contrast agents for cardiac and hepatic imaging because they reflect a characteristic echo due to nonlinear oscillation that can be detected using harmonic imaging (Lanka et al. 2007; Mulvagh et al. 2008). Their size and composition permits them to persist exclusively in the circulation and the microvasculature after intravenous injection. Recent observed bioeffects of microbubbles and ultrasound such as transient membrane permeabilization, microvascular leakage and endothelial cell death have stemmed interest in potential therapeutic applications using microbubbles such as gene therapy, localized drug delivery and thrombolysis (Unger et al. 2002). In the experiments here, microbubbles were utilized in combination with radiation, to enhance tumour response. The detailed biologic and molecular mechanisms underlying this novel technique of utilizing microbubbles to target the microvasculature is described in other work (Al-Mahrouki et al. 2010).

The purpose of the current study was to use high frequency ultrasound to detect early cell death caused by radiation and the novel use of ultrasound and microbubbles as radio-enhancing antivascular agents. This is a novel therapeutic approach that targets the tumour by directing the known bioeffects of microbubble agents toward a small region defined by a focused ultrasound beam. Ultrasound treatment was then combined with radiotherapy to treat prostate cancer xenografts *in vivo* to evaluate the potential enhancement of tumour cell death. Then, using quantitative high frequency ultrasound imaging, similar to that described in previous studies, evaluations of tumour responses after treatment were carried out. Ultrasound spectroscopic results were also compared with histopathologic specimens. We found an independent effect of ultrasound and microbubble treatments as a singular modality and, when combined with radiotherapy, there was also an enhancement of overall tumour cell death. Further, we found in this study that morphologic changes and cell death induced by the two treatments could be detected *in vivo* using quantitative high frequency ultrasound.