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Investigation of acoustic radiation force for radio-protecting normal tissues during radiation therapy

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Abstract— **Radiation therapy (radiotherapy) is the medical use of ionizing radiation as part of cancer treatment to erradicate malignant cells. Normal tissue tolerance is currently a major dose-limiting factor. As molecular oxygen plays a critical role in creating the radiation damage, we propose a novel approach, that is, the use of acoustic radiation force (ARF) to suppress the normal tissue oxygenation, for the purpose of protecting the normal tissue and increasing its tolerance during radiotherapy. This paper investigated the effects of ARF on tissue oxygenation. Both subcutaneous tissue and tumor were studied for comparison. Experiments have been carried out using a murine model. Preliminary results showed that ARF can effectively suppress normal tissue oxygenation, and at the same time had negligible effect on the tumor oxygenation. Further investigation is ongoing to characterize the time course of oxygen changes with different ultrasound parameters (frequency, intensity, ultrasound pulse duration, etc.), for the purpose of optimal control of tissue oxygenation.**

I. Introduction

Radiation therapy (or radiotherapy) is the medical use of ionizing radiation as part of cancer treatment to control malignant cells. Molecular oxygen is the best known mediator of radiation damage [1]. Studies in the literature [2-5] have shown that cells are much more sensitive to x-rays in the presence of molecular oxygen than in its absence (i.e. under hypoxia). The mechanism responsible for the oxygen effect is generally referred to as the oxygen-fixation hypothesis [1]. The free radicals (R) produced by indirect action in the critical target will react with oxygen to form RO_2, which is an organic peroxide (non-restorable form of target molecules), and will undergo further reaction ultimately to yield $ROOH$. This chemical change in target molecules constitutes a fixed damage. In the absence of oxygen, or in the presence of reducing species (for example, radioprotectors), the free radicals can react with H, thus restoring its original form [2].

In radiotherapy of cancer, normal tissue tolerance is a major dose-limiting factor [6]. During the past few decades several investigations have been directed toward increasing normal tissue tolerance by using some chemical compounds against radiation toxicity. The protective effect of these radioprotectors stems from their ability to scavenge free radicals, and facilitate the chemical restitution of the original target molecules. Several sulfhydryl compounds have been tested and found to be efficient against sparsely ionizing radiation, such as x- or γ-rays. Among these compounds, Amifostine is referred as the most effective radioprotector, and is currently the only radioprotective drug approved by FDA for use in radiotherapy. However, due to the issues relating to possible tumor protection and loss of therapeutic gain, radioprotectors have not been widely used in radiotherapy [2].

In this study, we propose a novel approach, that is, the use of acoustic radiation force to suppress the normal tissue oxygenation, for the purpose of protecting the normal tissue and increasing its tolerance during radiotherapy. Stationary sound waves have long been known to create banding effects in red blood cells in vivo [7-13]. Our hypothesis is that the acoustic radiation force could suppress the oxygen level in the normal healthy tissue, and at the same time had no or negligible effect on the tumor oxygenation. Preliminary experiments have been carried out using a murine model to investigate the effects of acoustic radiation force on the oxygenation. Both subcutaneous tissue and tumor were studied for comparison. Experimental methodology together with results and discussion is presented in the following sections.

II. Materials and Methods

2.1 Experimental setup

The experiment was conducted in a plexiglas water tank full of distilled water, which was autoclaved for about an hour before the experiment to remove ions and microbubbles to prevent cavitation and scattering of the acoustic field. The ultrasound signal was generated using a 1 MHz piezoelectric transducer (Channel Industries), which has a focal length of 7 cm. The ultrasound transducer was driven by a function generator (Agilent 33250A) together with an RF amplifier (Amplifier Research 25A250A), monitored using an oscilloscope (Tektronix TDS 2022). The ultrasonic field was measured and characterized using a hydrophone (Onda Co., HNR 500) prior to the experiment. A 2.5 cm thick piece of aluminum was used as the acoustic reflector and a 2.5 cm thick rubber block was placed behind the aluminum to absorb any spurious acoustic energy scattered by the tissue water boundary and in the side lobes of the focused ultrasound field (Fig. 1 (a)).

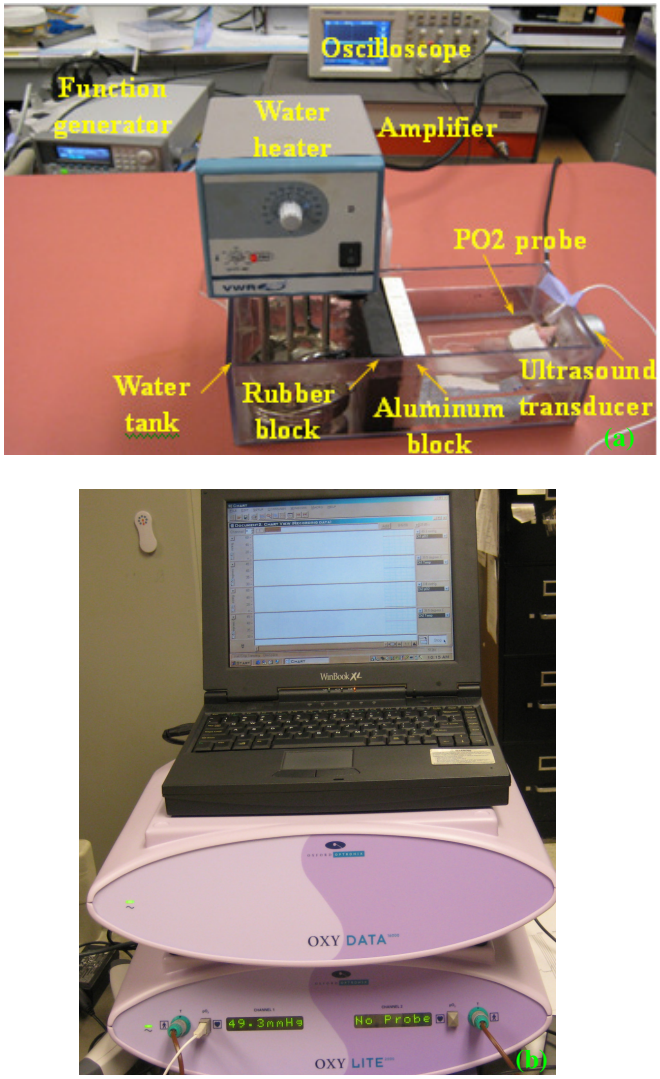

Fig. 1: Experimental setup: (a) setup for underwater pO2 measurement; (c) Oxylite system.

An oxygen measurement system, OxyLite 2000 (Oxford Optronix) was used for continuous quantitative monitoring of regional pO_2 in tissue. It includes a 30-gauge fiberoptic oxygen sensing probe and a data transferring control system. The probe consisted of a 230 µm diameter optical fiber for oxygen measurement and a thermocouple with fine wire leads for temperature monitoring. The oxygen probe tip had Ruthenium-III-(Tris)-chloride embedded in silicone polymer. It had a blue light-emitting diode that generated light pulses to induce fluorescence from the ruthenium luminophor. The detection system was based on the blue light excitation of ruthenium pigment, and this excitation was quenched by oxygen. Thus the fiber optic probe was most sensitive at low oxygen tension (<100 mmHg), such as found in tumors. The probe itself did not consume oxygen, which allowed the probe to remain in place for dynamic measurements. During the procedure, both temperature and pO_2 will be simultaneously measured and recorded by the OxyLite program (Fig. 1 (b)).

Several male NCR-NUM nude mice bearing human U87 glioblastoma (ATCC) xenografts on the right hind limb were used for the study. The xenograft-bearing mice were anesthetized initially with 100 mg/kg ketamine/0.3 mg/kg acepromazine and received a boost of 37.5 mg/kg ketamine/0.2 mg/kg acepromazine every 30 min. Our unpublished observations have shown that the combination of Ketamine and Ace Promazine has the least effects on hemodynamics. Thus, it was expected to have minimal effects on blood flow and tissue oxygenation.

2.2 Experimental procedure

1) Investigation of ARF effect on pO2 probe

The study of ARF effect on tissue oxygenation involved the direct measurement of the pO2 changes during the ultrasound procedure. Since ARF effect on the pO2 probe was unknown, experiment was first carried out using chicken skin to investigate the potential influence. Chicken skin was chosen as the experimental phantom because it was found to have a certain amount of residual pO2 within the detectible range (<100 mmHg) of the pO2 probe, which was unlikely to change during the ultrasound procedure in an ideal (noise-free) situation.

During the experiment, the chicken skin was placed at the ultrasound focus center on the platform inside the water tank, and the pO2 probe was inserted into it. At the start, the baseline measurement was taken for more than 10 minutes. Then ultrasound was administered in 10-second bursts, with 50-second relaxation periods between bursts. A total of five bursts were administered. After that, the measurement was continued for more than 15 minutes before stop.

2) Investigation of ARF effect on tissue oxygenation

During this experiment, the anesthetized mouse was put on a plastic holder and taped in place before positioned into the water tank. The fiberoptic probe was inserted into the tissue that was within the ultrasound focus center, and then taped in place, as shown in Fig. 1 (b). After that, water was filled in slowly into the water tank. The circulating water heater was turned on at a low speed to maintain the mouse's body temperature during this experiment.

Measurements were taken in the mouse on both the subcutaneous tissue and tumor, following the same procedure as was performed on chicken skin.

III. Results and Discussions

3.1 Results of ARF effects on fiberoptic oxygen probe

Figure 2 shows the pO2 readings in chicken skin during ultrasound pulses. As shown in this figure, the ultrasound pulses did not show significant effect on the pO2 readings. The small fluctuation of the pO2 as shown in this figure was due to the sensor noise. Compared to the changes caused by

ARF (as will be shown in the following section), it was negligible.

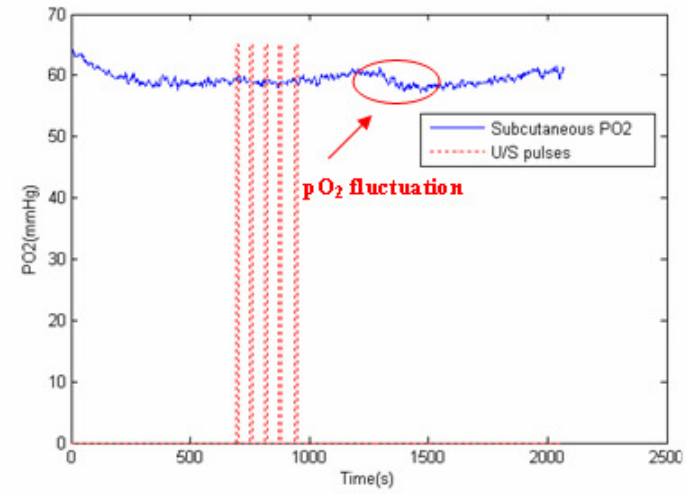

Fig. 2: pO_2 measurement in chicken skin during ultrasound pulses (vertical bars).

3.2 ARF effect on tissue oxygenation

The pO2 measurements in the subcutaneous tissue and tumor during ultrasound pulses were shown in Fig. 3. As demonstrated in Fig. 3 (a), the pO2 level in the subcutaneous tissue was stabilized at around 45 mmHg at the beginning. When the ultrasound was on, the pO2 level dropped from 45 mmHg to around 35 mmHg and stayed at that level for about 1000 s before returning back to 45 mmHg. The period that the oxygen level stays at the lowest is potentially useful for radiotherapy, since the normal tissue will be less radiosensitive with reduced oxygen level. The return of the pO2 level suggested that there was no permanent effect resulting from the ultrasound pulses. In contrast, there were no detectable changes in the tumor during and after the ultrasound pulses (Fig. 3 (b)). This suggests that ARF can be used in suppressing normal tissue oxygenation, without affecting tumor oxygenation.

Currently we observed the ARF effect in 3 mice. However, the patterns of pO2 changes, i.e. the onset time that the oxygen level started to decrease, the achievable lowest oxygen level, the dwell time that oxygen stayed at the lowest level, etc, varied in different mice under the same experimental protocol (ultrasound frequency, intensity, ultrasound pulse duration, etc). Further study with more mice, as well as different experimental protocols will be necessary to consolidate the findings, better understand the phenomenon, and characterize as well as optimize the control protocol, for the purpose of best protection of normal tissue during radiotherapy.

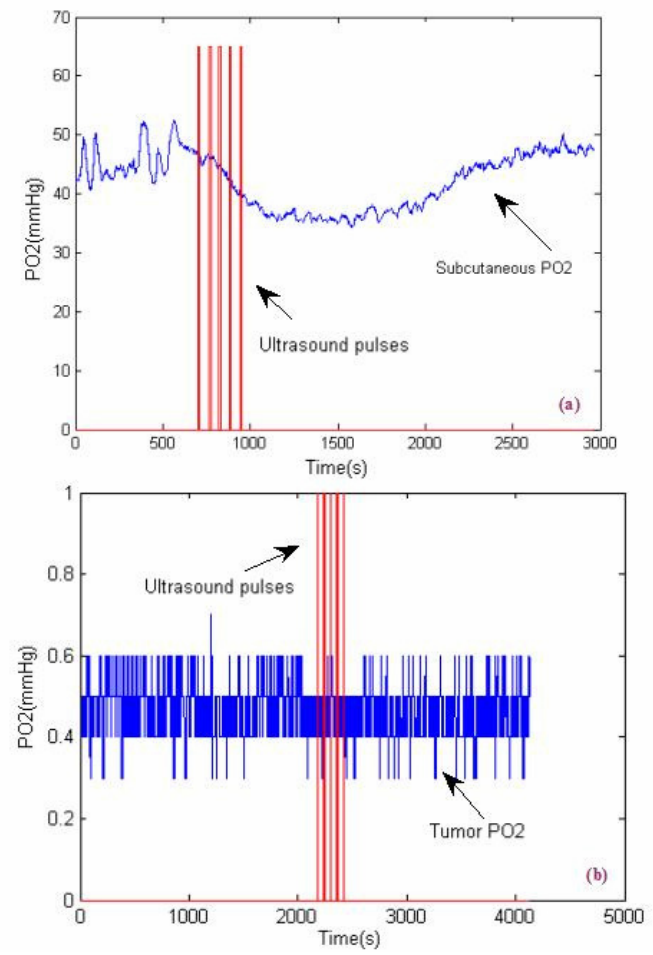

Fig. 3: ARF effects on (a) subcutaneous tissue and (b) tumor oxygenation.

IV. CONCLUSION AND FUTURE WORK

In this paper, we investigate the effect of ARF on tissue oxygenation for the purpose of radioprotecting the normal tissues during radiation therapy. Both subcutaneous tissue and tumor were investigated for comparison. Preliminary results showed that ARF can effectively suppress normal tissue oxygenation without affecting tumor oxygenation. In the future, we will conduct further studies to characterize the time course of pO2 changes with different ultrasound parameters (frequency, intensity, ultrasound pulse duration, etc.), for the purpose of optimal control of tissue oxygenation. Device for delivering the ARF during radiotherapy will be designed based on the study. We are also investigating the effectiveness of anti-angiogenic therapy in enhancing tumor oxygenation for better radiosensitivity. By combining anti-angiogenic therapy to improve tumor oxygenation prior to radiotherapy with ARF technique to protect normal tissue during the treatment, tissue oxygenation may be altered in a way that enhances the therapeutic ratio.

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