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Acoustic radiation force and optical spectroscopy for assessing tumor vessel normalization during anti-angiogenic therapy

Kaiguo Yan, Phyllis Wachsberger, Yan Yu

Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, PA

Abstract—**Molecular oxygen is the best known mediator of radiation damage during radiation therapy. This paper investigates techniques for enhancing tumor oxygenation by use of anti-angiogenic therapy for better radiosensitivity. A noninvasive monitoring technique, Acoustic radiation force (ARF)-induced optical spectroscopy, was proposed in this paper to track tumor oxygen changes during anti-angiogenic therapy. Male NCR-NUM nude mice bearing human U87 glioblastoma (ATCC) xenografts on the right hind limb were used for the study. An anti-angiogenic regimen using the drug AZD2171 (AsraZeneca) was found capable of improving tissue oxygenation during the first week of drug treatment. The optical spectroscopy technique was shown to be able to track tumor oxygenation changes during anti-angiogenic therapy, as verified by direct pO2 measurement. By using anti-angiogenic therapy to improve tumor oxygenation prior to radiotherapy, tissue oxygenation may be altered in a way that favors radiation therapy.**

I. Introduction

Ionizing radiation (IR) is currently an important treatment modality in a wide variety of malignant tumors. IR induces cell death by damaging DNA and other intracellular macromolecules. The best known mediator of IR damage is molecular oxygen [1]. The mechanism of oxygen's action in creating biological damage is related to free radical production following absorption of radiation by cells. Free radicals can interact with DNA damage to alter chemical structure and hence DNA metabolism, leading to cell death. Studies in the literature have shown that the response of cells to IR is strongly dependent upon oxygen [2-4]. Hypoxic cells are about three times more resistant to cell killing by radiation in comparison to well oxygenated cells. This can be illustrated in the survival curves for mammalian cells exposed to x-rays in the presence and absence of oxygen in B Palcic *et.al*. [5], which concludes that cells are much more sensitive to x-rays in the presence of molecular oxygen than in its absence (i.e. under hypoxia). For the same amount of radiation dose, well oxygenated cells can achieve higher level of cell killing.

It has been recognized that tumors are very heterogeneous with regard to oxygen levels and contain hypoxic micro-regions that are refractory to radiotherapy [6]. When tumor cells become hypoxic, they produce less free radicals following IR exposure, and therefore become more resistant to the effects of radiation. This suggests that enhancing tumor oxygenation can improve the level of cell killing, and thus lead to better radiation outcome.

The objective of this study is to enhance tumor oxygenation for better radiosensitivity and to detect the enhancement using a non-invasive method, ARF-induced optical spectroscopy. It has been reported that anti-angiogenic therapy can create tumor vessel normalization, through a process that prunes the immature vessels and fortifies the remaining ones, allowing for increased blood flow, oxygenation and improved chemotherapy and radiotherapy [7]. We have carried out experiments using a glioblastoma murine model to identify the onset and duration of tumor vessel normalization following anti-angiogenic therapy. Our hypothesis was that ARF-induced optical spectroscopy can be used to track tumor oxy/deoxy hemoglobin changes as a reflection of changes in tumor oxygenation. This technique was used in a previous study [8] to differentiate the tumor tissue from the normal tissue. The differentiation was based on the fact that the oxy/deoxy hemoglobin changes induced by ARF were primarily a phenomenon of normal vessel network morphology, and were not present under conditions of malignant, angiogenic growth. These changes were reflected in the simultaneous drops of the ratio of optical intensities (I) at two wavelengths, 560 nm and 540 nm (I560/540) during ultrasound pulses. In this study, the relative changes of the mean signals (I560/540) during anti-angiogenic therapy were examined to investigate its correlation with tissue oxygenation changes in the same mouse. Experimental methodology together with results and discussions is presented in this paper.

II. Materials and Methods

A. Experimental setup

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). During the experiment, the intensity of the ultrasound was maintained at Spatial Peak Temporal Average Intensity (ISPTA) ≈ 0.70 W/cm^2, which was below the FDA

therapeutic ultrasound limits (ISPTA=0.720 W/cm^2).

Diffuse reflectance spectra were collected using a fiber optic probe (Ocean Optics, R600-7-VIS/NIR), which consisted of a tight bundle of seven 600 μm optical fibers in a stainless steel ferrule with one collection fiber residing at the center of six illumination fibers. The collection fiber was connected using a 2048 pixel room temperature spectrometer (Ocean Optics, USB 2000-VIS/NIR) fitted with a grating for spectrum analysis between 200 nm and 1100 nm. The outer six illumination fibers were connected to a broadband halogen light source (Ocean Optics, HL 2000). Figure 1 (a) shows the ultrasound setup and optical spectroscopy system.

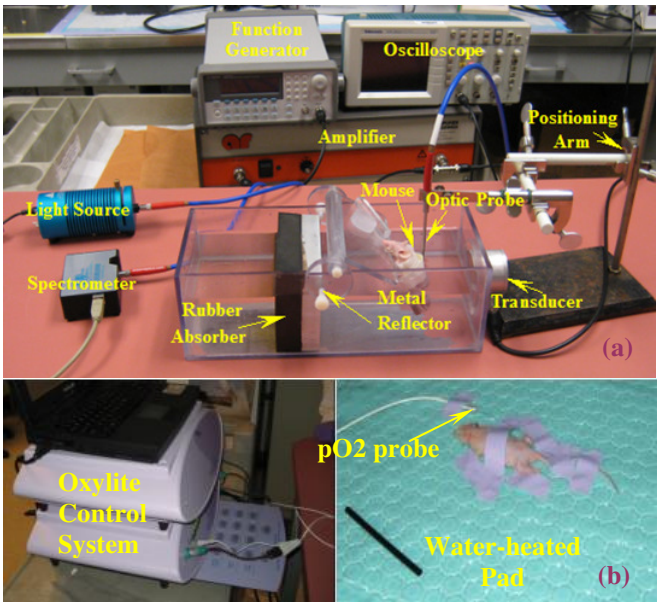

Fig. 1: Experimental setup: (a) ultrasound and optical spectroscopy system; (b) Oxylite system with a mouse on water-heated pad (blue).

An oxygen measurement instrument, OxyLite 2000 (Oxford Optronix), which had a 30-gauge fiberoptic oxygen sensing probe and a data transferring control system (Figure 1 (b)), was used for continuous quantitative monitoring of regional pO_2 in tissue. 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 oxymetry was based on the principle that the lifetime of the fluorescent pulse was inversely proportional to the oxygen tension in the tip. The temperature sensor (T-type thermocouple with polyurethane coating) was attached in close proximity to the oxygen probe tip to correct the temperature dependence of the fluorescence quenching. Since the detection system was based on the blue light excitation of ruthenium pigment and this excitation was quenched by oxygen, 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 were simultaneously measured and recorded by an OxyLite program.

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.

B. Experimental procedure

A total of 3 mice were used in this study, with one mouse observed for one week, and the other two observed for two weeks. Baseline measurements on the tumor legs were taken on the first day (Day 0) before injecting the drug into the tumors. ARF-induced optical spectroscopy measurements were taken first. During the experiment, the mice were positioned into the water tank, and the optical probe was fixed on the tumor legs using a positioning arm, ensuring contact but avoiding skin compression. Ultrasound was administered for 10-second bursts, with 50-second relaxation periods between bursts and a total of five bursts per position per experimental collection.

After that, the mice were placed on a temperature controlled water-heated pad, which can maintain the body temperature at 37 ± 0.5 °C during an experiment. Measurements were taken at the same locations where the optical measurements were taken. A guide hole was made first using a 25-gauge needle. Then the fiberoptic oxygen probe was inserted into the tissue through the hole with a depth around 2 mm, and then taped in place to avoid movement during the procedure. The Oxylite reading was taken for around 20 - 30 minutes in order to obtain a stable reading.

After that, the drug (AZD2171) was administered to the mouse every day with 3 mg/kg per day and measurements were taken every other day at the same location.

III. Results and Discussions

Figure 2 shows the measured pO_2 in the three mice over one week (for mouse 1) and two weeks (for mice 2 and 3) respectively. The mean values were examined here to remove the effects of the oxygen fluctuation during tissue metabolism, as well as sensor noise. Figure 3 shows the relative changes of pO_2 over the corresponding baselines in the three mice during the course of treatment. As shown in Figs. 2 and 3, in the first week, oxygen levels in all the three mice significantly increased on Day 4; while in the second week, after a large drop on Day 7, continuous drug treatments did not show effective improvement on the tumor oxygenation. Also, anti-angiogenic therapy exhibited slightly different effects on the individual mice during the course of treatment. For example, during the first week, pO_2 in mouse 1 was slightly improved from 1.6 mmHg on Day 0 (baseline) to 3.5 mmHg

on Day 2, then increasing to 15.9 mmHg on Day 4; while for mouse 2, pO_2 changed from 0.49 mmHg on Day 0 (baseline) to 0.21 mmHg on Day 2, then rising to 12.72 mmHg on Day 4; for mouse 3, pO_2 fluctuated from 0.14 mmHg on Day 0 (baseline) to 0.40 mmHg on Day 2, then increasing to 22.87 mmHg on Day 4. During the second week, continuous drug treatments did not improve the tumor oxygenation significantly: for mouse 2, pO_2 dropped from 2.12 mmHg on Day 7 to 0.50 mmHg on Day 9, and then slightly changed to 0.83 mmHg on Day 11; for mouse 3, there was a small improvement from 3.93 mmHg on Day 7 to 6 mmHg on Day 9, and then dropped to 4.96 mmHg on Day 11. In this study, pO_2 changes within 1 mmHg in the tumor were considered insignificant, since many factors can affect the readings. For example, the amount of oxygen in vessels fluctuated itself during tissue metabolism. In addition, the position of the pO_2 probe inside the tissue could also affect the readings. Thus to maintain the same insertion depth in the same mouse during the whole course of study was important for the purpose of comparison. The inaccurate probe position as well as sensor noise could also result in small changes of pO_2 readings during the experiments.

For the optical signals, the ratio I560/540 that correlated to the oxy/deoxy hemoglobin concentrations was examined here, which was found to be less than 1.10 in the hypoxia tumor tissue during the baseline measurement. After 3 days' anti-angiogenic therapy, the ratio increased close to 1.16 for mice 1 and 2, and 1.17 for mouse 3 (see Fig. 4). In the second week, the optical signal dropped, similar to the pO_2 measurements. Figure 5 showed the relative changes of the optical signal (I560/540) over baselines during the course of treatment.

The relationship between the optical signal changes and pO_2 changes was plotted in Fig. 6. As can be detected from this figure, the optical signal increased nearly monotonically with the pO_2 measurement. There was a small discrepancy in the optical signals and pO_2 measurement as marked in Fig. 6: in mouse 3, pO_2 measurement showed an improvement of 4.82 mmHg on Day 11, which was larger than the change of 3.79 mmHg on Day 7, while optical signal on Day 11 was changed by 0.04, which was smaller than the change of 0.06 on Day 7. This small discrepancy could be caused by the sensor noise, inaccurate positions of the pO_2 and optical probes, as well as tissue oxygen fluctuation. Since only large oxygenation improvement (>10mmHg) during anti-angiogenic therapy was of primary interests in this study, the discrepancy in tracking very small oxygen fluctuations was not of major concern.

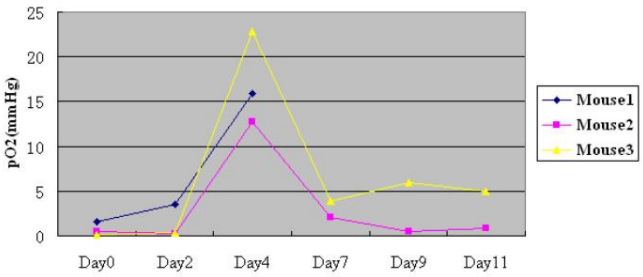

Fig. 2: pO_2 measurements.

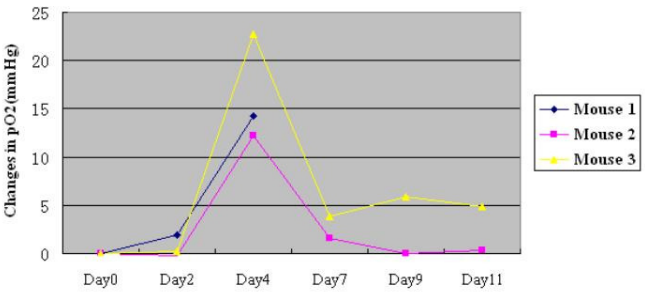

Fig. 3: pO_2 changes in three mice with reference to baseline measurement on Day 0.

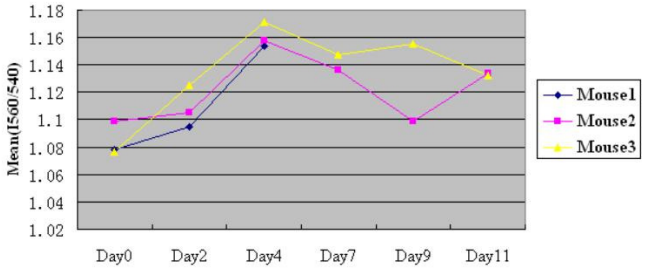

Fig. 4: Mean values of I560/540.

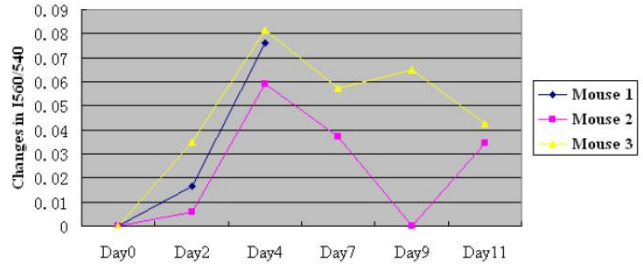

Fig. 5: Optical signal changes in three mice with reference to baseline measurement on Day 0.

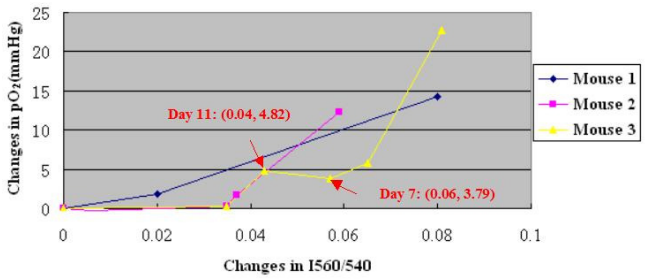

Fig. 6: Relationship between the optical signal changes and pO_2 changes.

ARF-induced optical spectroscopy was used during the experiment to produce another measure of the level of tumor vessel normalization in addition to the optical signals. In the well-oxygenated normal tissue, the ratio I560/540 would drop due to blood stasis caused by ARF[8]. However, this effect currently did not appear in the treated tumors during the course of anti-angiogenic therapy. This may be explained by the fact that the highest achieved tumor oxygen level in this study was about 23 mmHg, which was still much lower than the normal tissue oxygen level (generally ranging from 40 - 70 mmHg).

Overall, the optical signals (I560/540) exhibited a comparable pattern of change as the corresponding pO_2 measurements, as can be discerned from Fig. 2 to Fig. 6. Table 1 gave the correlation analysis of the optical signals and pO_2 measurements for the three mice. The critical values of the Pearson product-moment correlation coefficients (r) for one-tailed test at the 0.05 significance level were 0.988 for mouse 1 with 3-sample size, and 0.729 for mice 2 and 3 with 6-sample size [9]. The calculated correlation coefficients were larger than the corresponding critical values, which suggested the correlations of the optical signals and pO_2 measurements were statistically significant.

IV. CONCLUSION AND FUTURE WORK

In this study, results from current 3 mice have shown that tumor oxygenation was improved during the first week (the normalization window), while it dropped during the second week (reversal of normalization), which agreed with the findings reported in the literature [10]. This suggested that the optimal time for administration of radiation would be on Day 4 following the anti-angiogenic treatment. We also demonstrate that ARF-induced optical spectroscopy can be used as an effective non-invasive detection technique to track the tumor oxygenation changes during the therapy. Experiments are being carried out with more mice for further investigation. We will change the anesthesia to use Isoflurane gas based on veterinary advice, instead of Acepromazine +Ketamine, which is expected to have the minimum effects on blood flow and tissue oxygenation. Considering the potential radiation damage to the normal tissue, we are also investigating techniques to radio-protect the normal tissue during radiotherapy. By combining anti-angiogenic therapy to improve tumor oxygenation prior to radiotherapy with techniques to protect normal tissue during the treatment, we intend to alter tissue oxygenation in a way that favors radiation therapy.

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TABLE 1: CORRELATION ANALYSIS OF OPTICAL SIGNAL AND pO_2 MEASUREMENT

	Mouse 1	Mouse 2	Mouse 3
Correlation coefficient (r)	0.996	0.792	0.762