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Research Article

Clinical Measurements of Oxygen via Electron Paramagnetic Resonance (EPR) During and after Breast Radiation Therapy: Preliminary Results of Baseline Evaluations and Response to Hyperoxygenation

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- Breast cancer

Abstract

Purpose/Objectives: During radiation therapy (RT), patients often develop radiation-induced toxicities. EPR oximetry is an effective method for measuring oxygen levels. We present first-in-clinic data with sequential and repeated measurements of oxygen in normal breast tissue during and after whole breast radiotherapy.

Materials/Methods: Nine patients undergoing RT for breast cancer were enrolled in a pilot study for EPR oximetry. Normal breast tissue of patients were injected with the EPR reporter material prior to whole breast RT. Tissue oxygenation was assessed using a clinical EPR oximeter. Patients were measured every week during RT and every 2-3 month follow-ups using EPR oximetry.

Results: All patients, an average of 8-9 measurements was taken for a total of 73 measurements across patients. During their RT, the average baseline pO_2 value across patients was 7 ± 3mmHg. When hyperoxygenation was applied, there was a statistically significant rise of 27mmHg ($p = 0.005$). Following completion of RT, the baseline and hyperoxygenation values were 11 ± 2mmHg and 25 ± 6 mmHg, respectively ($p = 0.01$). For both baseline and hyperoxygenation, no significant difference was observed between measurements taken during and after radiation therapy ($p>0.1$).

Conclusions: Our preliminary data from nine patients have validated the feasibility and reproducibility of EPR oximetry to measure temporal changes in the oxygenation of normal breast tissue during and after an RT course. The results indicate that EPR oximetry may be used to in clinical trials to investigate oxygen levels and their response to potentially hyperoxygenation interventions, which could be very useful in determining the clinical efficacy of radiosensitization and the mechanism of fibrogenesis.

ABBREVIATIONS

RT: Radiotherapy; EPR: Electron Paramagnetic Resonance

INTRODUCTION

Our group has developed and applied the first clinical method to make direct repeated measurements in human subjects [1]. While the principal clinical motive for these developments has been to enhance radiation therapy and to combine therapies by providing direct information on oxygen levels [2], another motive for utilizing these unique capabilities is to study the mechanisms of radiation therapy side effects, namely normal tissue toxicity. Specifically, as reported in this study, this measurement technique is being used to determine whether radiation therapy has a mechanistic role in the level of oxygenation in normal tissue. The pathophysiological consideration that makes this mechanism plausible is the consensus that the long term fibrosis seen after irradiation is due in major part to changes to the fine vasculature

[1,3]. The unique capabilities of clinical EPR oximetry now make it feasible to test the hypothesis that radiation induced hypoxia is a predictor of long term fibrosis.

In this preliminary study, an EPR oximetry sensor (Carlo Erba Ink) was injected into the normal breast tissue of patients who had undergone lumpectomy and were scheduled to be irradiated (with or without also receiving adjuvant chemotherapy or hormonal therapy). Using the permanently located oxygen sensor, pO_2 levels were repeatedly measured to evaluate changes in oxygen levels in tissue over a several month timeframe, i.e., during and after radiation therapy. The technique for making each measurement also allowed continuous data collection over a short term (several min) period. The total time for each measurement (~30 minutes) was divided into three approximately equal periods, first while the patient was breathing normal room air (baseline), during breathing enriched oxygen (hyperoxygenation) and after return to breathing room air (recovery). The intervention with enriched oxygen provides considerable additional information on the pathophysiological state of the tissue, reflecting changes associated with the delivery and utilization of oxygen by the tissues within a short timeframe. This information provides the potential for determining whether radiation oncologists in the future could intervene, e.g., by adding a hyperoxygenation treatment during each fraction of radiotherapy, to enhance radiosensitivity of hypoxic tumors and reduce potential oxidative damage to normal tissues [2].

MATERIALS AND METHODS

Patients

All patients reported here were enrolled in an oximetry study using electron paramagnetic resonance (EPR) where Carlo Erba ink was used as the oxygen sensor. Potentially eligible patients were referred to Emory Radiation Oncology for radiotherapy following surgery for breast cancer and were screened initially by the primary study radiation oncologist and clinical research coordinator to be sure they satisfied the study inclusion and exclusion criteria. Patients passing the initial screening and those who agreed to be approached about participating in a clinical study were then informed about the study. Eligible patients agreeing to participate were asked to sign a written informed consent for the study. The study was an observational study, i.e. it did not involve any study treatment or intervention that was expected to change the outcomes of their usual treatment. The usual care for eligible patients required that they had undergone surgical removal of the tumor (either lumpectomy or mastectomy) and were scheduled to receive adjuvant radiation therapy; patients may also have received adjuvant hormonal therapy (anastrozole and/or tamoxifen) and/or chemotherapy (taxotere and cytoxan or taxol and herceptin) during the study.

During the enrollment procedure, all consenting patients were injected with the Carlo Erba ink in the area of the breast outside of the surgical scar but well within the planned radiation field. All consented patients were asked to agree to participate in approximately weekly oximetry measurements occurring during their radiation treatment and occasionally during follow-ups afterward. All also agreed to be measured periodically for signs of fibrosis by study personnel using ultrasound and other skin measurement techniques. This preliminary report focuses on the EPR oximetry data, since not enough time has elapsed post radiation to determine whether they have developed fibrosis.

The basic information about demographics and medical data for all nine patients enrolled in this study are reported in Table 1. Patient age ranged from 36-65; two-thirds were black; the average duration of being measured by EPR was 9.5 months; all but two patients had also had adjuvant hormonal therapy during the period when being measured. Not detailed in the Table, all nine women had had a lumpectomy; all received fractionated radiation therapy to the whole breast (range 42.5 Gy to 50 Gy) and two thirds received a boost of 10 Gy.

EPR Oximetry and Calibration

To directly measure the pO2 levels in the local region, sterile paramagnetic oxygen-sensing micro particulates of carbon suspended as an ink were injected subcutaneously into the region of the breast to be irradiated but not involving the surgical scar. While the carbon particulates used in oximetry have been chosen to ensure they are paramagnetic and therefore can be detected by EPR, they are similar to the black inks used in tattoos and in medical practices for marking [1]. More specifically, the ink injected into patients in this study consists of 50µl of a charcoal suspension (Carlo Erba®, 100mg/ml in saline also containing 3% Arabic gum to make it more viscous and reduce diffusion). It is

Table 1: Patient Demographics, EPR oximetry measurements, and Additional Adjuvant Therapy.

Patient ID	Age	Race	No. EPR Measurements	Total weeks between first and last EPR measurement	Weeks receiving Adjuvant Hormonal Therapy during EPR measurements
10083	65	White	11	48	Hormone – 44
10084	53	Black	13	47	Hormone - 41
10086	53	Black	14	48	Hormone - 48
10087	52	Black	14	45	None
10088	62	White	8	14	None
10089	49	Black	13	43	Hormone – 43*
10090	36	Black	8	42	Hormone - 28
10091	60	Black	12	34	Hormone – 24*
10092	47	White	11	23	None

*received chemotherapy before radiation therapy and EPR measurements

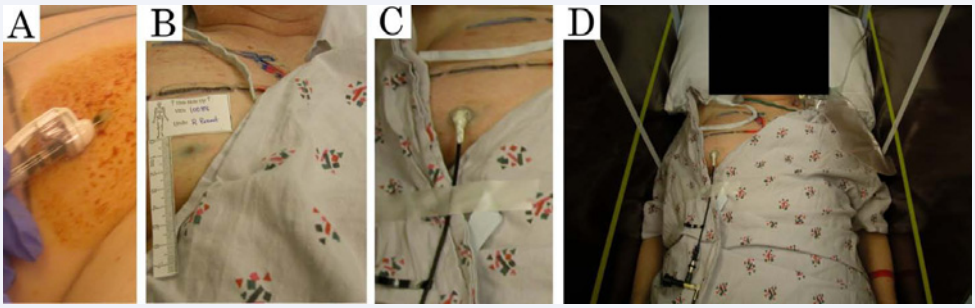

Figure 1 Ink injection, resonator placement, and measurement conditions. The figure shows: (A) patient being injected with the Carlo Erba India ink probe, (B) post-injection view of the tattoo, (C) attachment of EPR resonator on the ink probe, and (D) the measurement of the patient in the EPR oximetry machine.

administered using a 0.5ml insulin syringe with a 29G needle (Figure 1A). After the initial injection of the Carlo Erba (CE) ink, the carbon particulates can be used repeatedly and noninvasively to measure the subcutaneous pO_2 levels (Figure 1B).

After a suiTable time, allowing for any local inflammatory response to the injection to dissipate, in vivo oximetry measurements can be made entirely non-invasively using a specially designed clinical EPR spectrometer. The principal parts of the spectrometer consist of a 420 G permanent magnet, a custom-made microwave bridge operating at a low frequency (1.2 GHz), and a flexible surface loop resonator (Figure 1C and Figure 1D) [2].

The linewidth of the EPR spectrum can be related to the oxygen level using a calibration curve such as shown in Figure 2. It should be noted, however, that the calibration curve is made under carefully controlled conditions in vitro. It is possible that when the ink is in tissue, which can have different geometric distribution, its response to oxygen may not be fully represented by the calibration curve. Therefore, the most valuable data for clinical insights may be variations in patients regarding patterns of changes in oxygen measurements over time and in response to hyperoxygenation during a measurement session. As discussed elsewhere [5,6] there is no single absolute value for oxygen in a real tumor or other tissues because of the heterogeneity in oxygen over space and time. Therefore the focus of this paper is on the changes in the same patient and tissue over time and in the response to breathing hyperoxygenated gas.

Measurements and Visits

EPR measurements were taken following the initial visit that included the injection of the EPR oximetry probe. Once the initial injection (tattoo) was complete, each patient was measured before her first treatment, each week of her radiation therapy

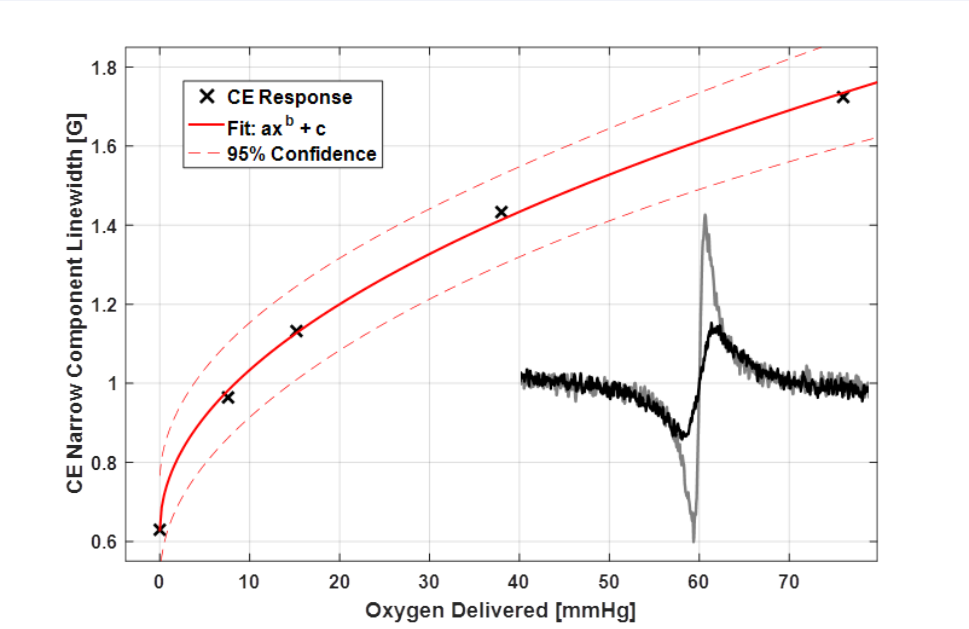

Figure 2 EPR calibration curve. This figure shows the Carlo Erba's EPR linewidth response (see X's) based on the amount of oxygen applied in a model system. The red line is the regression curve, or the fit, of the expected CE linewidth based on the oxygen applied. The black and gray signals on the bottom right corner show the narrow component of the CE linewidth of high and low oxygen concentration respectively.

regimen, and from one to three months following the completion of her treatment. During each visit, each patient was placed supine on the clinical EPR spectroscopy Table and the tattoo and surrounding area was cleaned with an alcohol wipe. Afterwards, the flexible resonator was attached to the tattoo using a fixation ring and occasionally some medical tape was used to support the adherence of the resonator to the tattoo (Figure 1C). Once the resonator was properly fixed, the patient was then pushed in between the permanent magnets with the aim of positioning the tattoo central to the magnetic field (Figure 1D). Afterwards, the resonator was attached to the coupler and the measurement process can begin. When all the measurement equipment and the patient were in place, the clinical EPR spectroscopy machine was turned on, calibrations was applied, and measurements were taken for ~ 30 minutes as described below.

Each measurement is comprised of three phases: the baseline phase, hyperoxygenation phase, and recovery phase. Each phase contains 7-10 sets of measurements, where each set accounts for the median pO_2 value in the tissue for one minute. During the baseline phase, the patient is free-breathing normal air for about 8-10 minutes. Then the patient puts on a non-rebreather mask and starts breathing 100% oxygen that is fed at a rate of 15L/min for 10 minutes in the hyperoxygenation phase. Finally, the patient takes off the non-rebreather mask and continues the rest of the measurement free-breathing normal air in the recovery phase.

RESULTS

Example measurement of a patient

Within one measurement (Figure 3), it is observed that during the baseline phase, the pO_2 remains fairly constant. Then during the application of 100% oxygen in the hyperoxygenation phase, the pO_2 levels rise. Some patients quickly reach an equilibrium like patient 10084 and the pO_2 value plateaus, while others, like patient 10092, do not equilibrate over the hyperoxygenation phase. When the patient returns to breathing room air, the pO_2 is observed to decrease to approximately the baseline values within a few minutes.

Multiple measurements of a patient

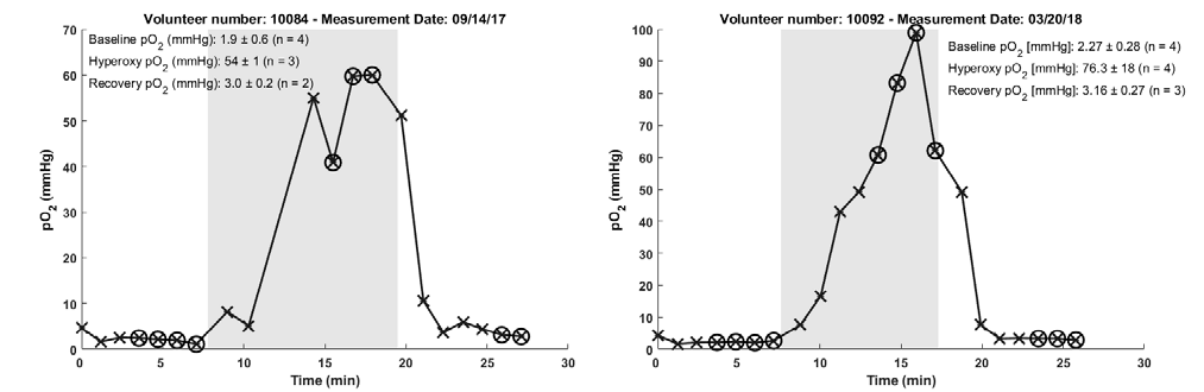

Figure 3 A single EPR measurement session for two patients. For each measurement session, sets of pO2 values are shown in Xs. The gray section indicates hyper-oxygenated gas breathing. The circles indicate the sets that were selected to calculate the mean and standard deviation of the pO2 in each period; these means were used to compare across measurement sessions. As expected, the selected sets were relatively stable toward the end of each period while breathing room air but are fairly unstable at the end breathing hyper-oxygenated air.

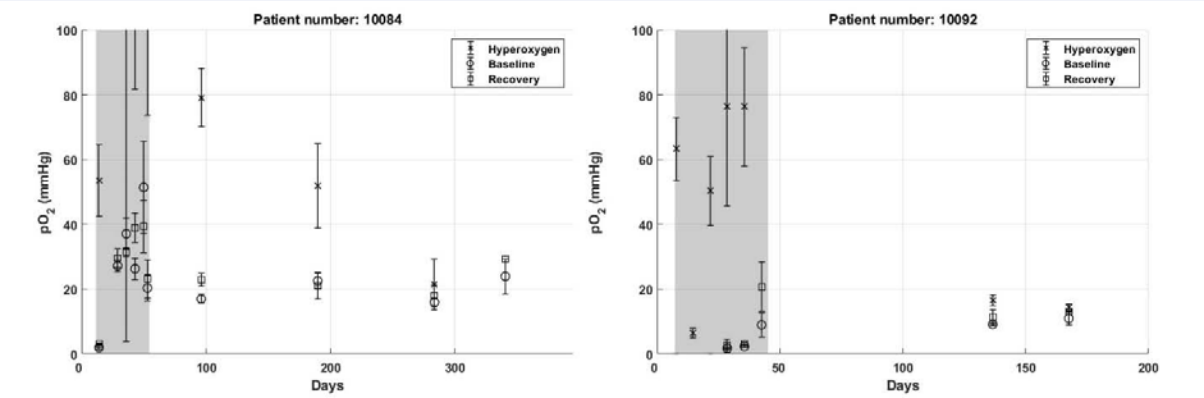

Figure 4 Multiple Measurement Sessions for Individual Patients. For each visit, the Os, indicate the mean and SD during the initial period while breathing room air. The Xs indicate the mean and SD during hyperoxygenation. The square indicates the mean and SD after returning to breathing room air. All means and SDs were calculated as shown in Figure 3. Day zero refers to the date of patient ink injection. The gray area indicates the duration of the radiation treatment. The pO2 range was limited to 100mmHg for the sake of visualization.

In all nine patients we were able to make repeated measurements over time using the usual protocol. To date we have measured for as long as 18 months after the original injection. Presumably the measurement could continue indefinitely; in studies using India Ink in the foot we have followed the same subject for more than 10 years [1]. Across measurements for patients (Figure 4), it can be observed that there is a clear separation, i.e., a statistically significant difference, between the baseline and hyperoxygenation pO_2 values that is observed via EPR oximetry in human breast tissue before and after radiation therapy.

All measurements across patients

The baseline average, hyperoxygenation average, and the difference between the two (delta pO_2) for all measurements across all patients are reported in Table A in the appendix. An analysis of the all measurement values is given in Table 2 and the comparison of different periods; during RT vs. post RT and baseline vs. hyperoxygenation is given in Table 3.

Table 3 shows that there was a significant short term increase in pO_2 levels observed between the baseline and hyperoxygenation (during 100% oxygen breathing) in the normal breast tissue that was being irradiated (these values' combined measurements made pre, during and post radiation therapy). However, looking at trends over the long-term, especially comparing measurements taken during versus post radiation therapy, there were no significant differences in average pO_2 observed between measurements taken while undergoing active radiation therapy versus post radiation, for either the baseline or hyperoxygenation averages.

DISCUSSION

Our preliminary data from nine patients have validated the feasibility and reproducibility of EPR oximetry to measure temporal changes in the superficial oxygenation of normal breast tissue during and after a radiation therapy course. In addition, evidence from other studies involving healthy volunteers and patients suggest that EPR oximetry using India ink as a pO_2 reporter is viable for prolonged periods of time (observed now, over 10 years) and can be used successfully in a variety of superficial normal and tumor tissues [4], which highlight the potential versatility of EPR oximetry in many applications.

The patients were observed to respond significantly to hyperoxygenation both during and after radiation therapy as

Table 2: Analysis of all measurements.

Patient ID	Measurement Period	Average baseline pO2 [mmHg]	Average hyper-oxygenation pO2 [mmHg]	Average delta [mmHg]	Average delta for period [mmHg]	SD across deltas for Period [mmHg]
10083	Pre-RT	n/a	n/a	n/a	2.4	n/a
10083	During-RT	-0.7	37.2	n/a	37.8	45.9
10083	<3mo FU	10.6	12.9	2.3	3.1	n/a
10083	>3mo FU	n/a	n/a	n/a	2.1	1.6
10084	During-RT	27.3	99.3	n/a	76.7	43.1
10084	<3mo FU	19.8	50.9	32.4	63.3	n/a
10084	>3mo FU	n/a	n/a	n/a	17.0	21.7
10086	During-RT	-0.3	22.8	n/a	23.1	20.9
10086	>3mo FU	11.4	26.2	14.2	14.2	17.4
10087	During-RT	6.4	14.7	n/a	6.0	12.5
10087	>3mo FU	8.0	11.0	3.8	3.8	0.9
10088	During-RT	0.0	4.7	n/a	4.6	8.2
10088	<3mo FU	2.2	6.5	4.3	4.3	n/a
10089	During-RT	2.7	8.7	n/a	6.0	4.3
10089	>3mo FU	6.6	13.7	9.3	9.3	12.1
10090	During-RT	4.1	23.7	n/a	19.5	16.3
10090	<3mo FU	17.1	62.5	45.4	85.6	n/a
10090	>3mo FU	n/a	n/a	n/a	5.3	n/a
10091	During-RT	21.9	36.0	n/a	14.1	10.4
10091	<3mo FU	16.7	27.1	10.4	9.0	n/a
10091	>3mo FU	n/a	n/a	n/a	10.8	3.1
10092	During-RT	1.9	58.8	n/a	58.3	30.5
10092	<3mo FU	10.0	15.8	5.8	7.7	n/a
10092	>3mo FU	n/a	n/a	n/a	3.8	n/a

*for n/a values, there were not enough measurements in that period to calculate average values.
**FU – follow up

Table 3: A summary of all measurements.

	Baseline	Hyperoxygenation	p-value
During-RT	7 ± 3 (SD = 10, n = 9)	34 ± 10 (SD = 30, n = 9)	0.005
Post-RT	11 ± 2 (SD = 6, n = 9)	25 ± 6 (SD = 19, n = 9)	0.012
p-value	0.115	0.354	

*SD – standard deviation

shown in Table 3. To date, no significant differences in pO_2 were observed between measurements made during and measurements made after the course of radiation therapy. This may be due to the small patient size and/or the length of time over which the measurements were made. In a per-patient basis, some patients (pt10083, 10084, and 10092) were observed to respond more significantly to hyperoxygenation in the short term (during RT); while most patients responded with a decrease in response to hyperoxygenation in the long term with the exception of pt10090 where the response to hyperoxygenation in the midterm (after RT, under 3mo follow-up) increased (as reported in the appendix). This is an interesting observation that may suggest that the response to oxygen for normal tissue decreases over time, perhaps due to radiation induced fibrosis. The intention is to continue to follow these patients and to analyze ultrasound images to determine if there is a correlation between changes in oxygen response and the occurrence of fibrosis as observed by ultrasound. Additionally, we will examine potential relationships among trends in baseline, responses to hyperoxygenation, and ultrasound measurements.

CONCLUSION

This report represents the first study in which oxygenation changes have been measured via EPR in normal tissues over a long period in patients receiving therapeutic levels of ionizing radiation, making measurements in the human breast during and after radiation therapy. While the data are not yet available (due to the long period of development of the problem) to draw any conclusions about the potential etiology of hypoxia and subsequent development of fibrosis in response to radiation, the results indicate that such studies now are quite feasible. Additionally, it is suggested that with the combination of 100% oxygen breathing and EPR oximetry, we can observe significant dynamic effects on pO_2 levels in human subjects which may provide another dimension with which to evaluate the oxygenation of tumors targeted by radiation. In the future, we will further explore the relationship between the change in pO_2 levels and radiation induced fibrosis and will expand the cohort to include different sites, interrogating superficial tumor beds in conjunction with irradiated normal tissue.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

ABF and HMS are co-owners of Clin-EPR, LLC of Lyme NH that manufactures EPR spectrometers for clinical and preclinical investigational use only.

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