Temporal variation in the response of tumors to hyperoxia with breathing carbogen and oxygen

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Abstract

The effect of hyperoxygenation with carbogen (95% O_2 + 5% CO_2) and 100% oxygen inhalation on partial pressure of oxygen (pO_2) of radiation-induced fibrosarcoma (RIF-1) tumor was investigated. RIF-1 tumors were innoculated in C3H mice, and aggregates of oximetry probe, lithium phthalocyanine (LiPc), was implanted in each tumor. A baseline tumor pO_2 was measured by electron paramagnetic resonance (EPR) oximetry for 20 minutes in anesthetized mice breathing 30% O_2 and then the gas was switched to carbogen or 100 % oxygen for 60 minutes. These experiments were repeated for 10 days. RIF-1 tumors were hypoxic with a baseline tissue pO_2 of 6.2−8.3 mmHg in mice breathing 30% O_2. Carbogen and 100% oxygen significantly increased tumor pO_2 on days 1 to 5, with a maximal increase at approximately 32−45 minutes on each day. However, the extent of increase in pO_2 from the baseline declined significantly on day 5 and day 10. The results provide quantitative information on the effect of hyperoxic gas inhalation on tumor pO_2 over the course of 10 days. EPR oximetry can be effectively used to repeatedly monitor tumor pO_2 and test hyperoxic methods for potential clinical applications.

Key words: carbogen; electron paramagnetic resonance; hyperoxia; multi-site electron paramagnetic resonance oximetry; oximetry; oxygen; partial pressure of oxygen; radiation-induced fibrosarcoma

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INTRODUCTION

The efficacy of any treatment modality is largely influenced by the tumor microenvironment. One of the most important factors that influence the treatment outcome is the existence of hypoxia in solid tumors. It has been shown that hypoxia reduce the efficacy of radiation treatment, surgery and chemotherapy (Fyles et al., 1998; Knocke et al., 1999; Kaanders et al., 2002c; Nordsmark et al., 2005). A direct correlation between the oxygen levels in the tumors and treatment outcome has been reported (Nordsmark and Overgaard, 2000; Moeller et al., 2007; Kappler et al., 2008; Lovey et al., 2008; Rockwell et al., 2009). Consequently, an increase in the oxygen levels can improve the treatment outcome of tumors (Rudat et al., 2000; Robinson et al., 2001; Schuuring et al., 2002, 2006). Carbogen (95% O_2 + 5% CO_2) and 100% oxygen are the most convenient methods as they involve direct inhalation and do not inflict any toxicity (Overgaard and Horsman, 1996; Kulkarni et al., 2007). Breathing carbogen in combination with nicotinamide administration was used to improve tumor response to accelerated radiotherapy in head-and-neck tumors (Saunders et al., 1997; Kaanders et al., 1998, 2002b; Bernier et al., 2000; Janssens et al., 2012). However, only a poor to modest improvement in the treatment outcome was observed in the clinical trials (Bernier et al., 2000; Kaanders et al., 2002a). This is likely

due to a lack of knowledge about the oxygen levels in the tumors during treatments with hyperoxic approaches.

Techniques that can provide direct and repeated measurements of tumor partial pressure of oxygen (pO_2) will be extremely useful to detect therapeutic window during which the tumors response to hyperoxic strategies and then schedule radiotherapy to improve treatment outcome. The oxygen electrode technique has been considered the gold standard for assessing tumor oxygenation in the clinic (Evans and Koch, 2003; Milosevic et al., 2004; Williams et al., 2005). This approach provides a direct and rapid measurement of tissue pO_2, but use of the electrodes has a number of limitations. These include their applicability only to easily accessible tumors and the failure to distinguish necrosis from hypoxic viable tissue. Furthermore, oxygen electrodes cannot provide repeated measurements of temporal changes in pO_2 that is vital in determining the efficacy of anticancer treatments (Evans and Koch, 2003; Milosevic et al., 2004; Williams et al., 2005). Methods based on nuclear magnetic resonance (NMR) principles also have been developed such as ^{19}F-NMR spectroscopy (Hunjan et al., 2001), blood oxygen level dependent (BOLD) imaging (Baudelet and Gallez, 2002), and Overhauser methods (Krishna et al., 2002). While these methods have provided useful data and have the benefit of widely available instrumentation, each has a limitation for *in vivo* applications. ^{19}F-NMR spectroscopy requires the injection of the probe directly into the tumor and therefore has limited ability to make repeated measurements during therapy. BOLD imaging does not require any injection, but the information that it provides, the total amount of deoxyhemoglobin in the blood, cannot be related directly to the oxygen levels in the tumor. Overhauser method also relies on the repeated injection of paramagnetic material and has limited sensitivity. *In vivo* electron paramagnetic resonance (EPR) oximetry has the ability to provide repeated measurements of tumor pO_2 to test and optimize strategies designed for hyperoxygenation and demonstrate therapeutic benefits (Hou et al., 2004, 2007, 2009, 2010, 2011, 2013; Khan et al., 2009, 2010). We have systematically investigated the effect of breathing carbogen and 100% oxygen on the pO_2 in subcutaneous radiation-induced fibrosarcoma (RIF-1) tumors in the experiments repeated for up to 10 days. The maximum increase in tumor pO_2, time to reach maximum pO_2 (T_{max}) and the percentage of tumors that showed a significant increase in pO_2 were used to evaluate the effectiveness of breathing carbogen and 100% O_2 inhalation for hyperoxia.

MATERIALS AND METHODS

Animals and tumor models

All animal procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Dartmouth Medical School (Geisel School of Medicine). The RIF-1 cells were a gift from Dr. J. B. Mitchell's laboratory at the National Cancer Institute. This well-established subcutaneous tumor model has been used for several studies in our laboratory (Hou et al., 2004, 2007, 2009, 2010, 2011). The cells were cultured *in vitro* in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), glutamine and antibiotics. The procedure for tumor inoculation has been described previously (Hou et al., 2004, 2007, 2009, 2010, 2011). Briefly, female C3H/HEJ mice (18–20 g, Charles River Laboratories, Wilmington, MA, USA) were anesthetized (1.2% isoflurane, 30% O_2), and a suspension of $2-3 \times 10^5$ RIF-1 cells in 100 μL was injected subcutaneously into the left posterior flank. The tumors reached a size of 150–200 mm^3 in approximately 12–14 days post cell inoculation.

Implantation of the lithium phthalocyanine (LiPc) oximetry probe

The LiPc crystals were synthesized in our laboratory and the physico-chemical properties of LiPc crystals have been described previously (Liu et al., 1993; Swartz and Walczak, 1998). The LiPc crystals have a single sharp EPR line whose width is highly sensitive to pO_2. The EPR spectra reflect the average pO_2 on the surface of each LiPc aggregate (30–50 μg with an approximate volume of 0.09–0.15 mm^3) injected in the tumors. The procedure for LiPc injection has been described earlier (Hou et al., 2009, 2011, 2013). Briefly, the mice were anesthetized (1.5% isoflurane with 30% O_2), and two aggregates of the LiPc crystals were implanted at a depth of 2 mm and at a distance of 4 mm into each tumor using 25-gauge needles. A baseline tumor pO_2 were measured 24 hours after LiPc implantation for 30 minutes in anesthetized mice (1.5% isoflurane with 30% O_2) by using multi-site EPR oximetry described below. The tissue pO_2 measured from two LiPc implants in each tumor were pooled to determine average pO_2 on each day (Hou et al., 2009, 2011, 2013).

Experimental groups

The mice were randomly assigned into six groups which were allowed to breath normobaric carbogen (group A) or 100% O_2 (group B) multiple times on days 1–5 and day 10 or breathing carbogen once on day 1 (group C), day 3 (group D), day 5 (group E) and day 10 (group F) for 60 minutes after 30 minutes of baseline pO_2 measurements in anesthetized mice breathing 30% O_2 (**Table 1**).

Multi-site EPR oximetry

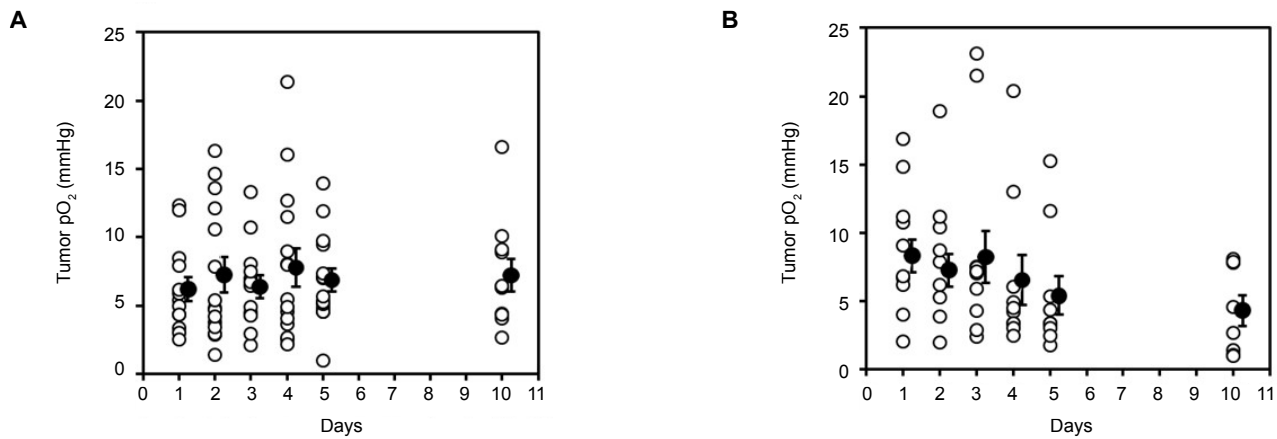Assessment of tissue pO_2 at 2–4 sites simultaneously and repeatedly by EPR oximetry has been used in animal models

A

B

Figure 1: Baseline RIF-1 tumor pO$_2$ over days.
Note: (A) Group A, carbogen, n = 12–16 implants at each time point; (B) group B, 100% O$_2$, n = 8–10 implants at each time point. "○" refers to tumor pO$_2$ recorded from each LiPc aggregate and "●" means tumor pO$_2$ obtained by pooling the pO$_2$ values of both LiPc aggregates in each tumor measured for 20 minutes. pO$_2$: Partial pressure of oxygen; RIF: radiation-induced fibrosarcoma; LiPc: lithium phthalocyanine.

Table 1: Experimental groups			
Group	Animal number	Intervention	Day(s) administered
A	9	Carbogen	Days 1–5 and day 10
B	5	100% O$_2$	Days 1–5 and day 10
C	10	Carbogen	Day 1
D	11	Carbogen	Day 3
E	6	Carbogen	Day 5
F	10	Carbogen	Day 10

(Hou et al., 2009, 2011, 2013). The pO$_2$ measurements were performed on an L-band (1,200 MHz) EPR spectrometer designed for *in vivo* experiments (Swartz and Walczak, 1998). A set of coils capable of generating a magnetic field gradient in the Z-direction with a magnitude up to 3.0 G/cm was used to separate the EPR spectra of LiPc implants in each tumor (Smirnov et al., 1993). The spectrometer parameters were: incident microwave power, 0.8–8.0 mW; magnetic field center, 425 gauss; scan range, 2 gauss; modulation frequency, 24 kHz; modulation amplitude was one-third of the EPR line width with scan time of 10 seconds. The EPR line-widths were converted to pO$_2$ using a calibration determined for each batch of LiPc crystals (Liu et al., 1993; Hou et al., 2009, 2013).

Physiological control and histological analysis
During EPR oximetry measurements, the body temperature of the animals was monitored using a rectal probe and animals were maintained at 37.0 ± 0.5°C using a thermostatically controlled heated pad and a flow of warm air.

At the end of experiments, the animals were euthanized, tumors removed, fixed, and sectioned. Microscopic examination (hematoxylin-eosin staining) of the tissue around the implanted LiPc deposits was performed to assess the tumor micro-environment.

Statistical analysis
A paired *t*-test was used to determine the statistical significance of the changes in pO$_2$ and tumor volume within the group and an unpaired *t*-test was used to determine the significance between groups. The paired comparison reduces the effects of animal to animal heterogeneity and eliminates differences of the baseline pO$_2$. The chi-square test was used to compare the percentage (%) of tumors with an increase in pO$_2$ of more than 50% from the baseline during first 20 minutes of breathing carbogen or oxygen compared to day 1. The multilevel linear mixed effects model was used to analyze the longitudinal pO$_2$ data in the log scale (Demidenko and Stukel, 2002; Demidenko, 2004). An exponential quadratic function of time was used to determine maximum pO$_2$ (pO$_{2max}$) and T$_{max}$ during carbogen and 100% oxygen inhalation. Each curve was analyzed accounting for two sources of variation: inter- and intra-mouse variations. Calculations were done by the statistical package S-Plus version 6.2 (Insight Inc., Seattle, WA, USA). All data of mean baseline and changes in pO$_2$ including pO$_{2max}$ and tumor volume are expressed as the mean ± SEM. The tests were two-sided, and a change with $P < 0.05$ was considered significant. n is the number of the implant, N is the number of animals in each group.

RESULTS
Effect of breathing carbogen or 100% O$_2$ on tumor pO$_2$ and T$_{max}$
Figure 1A and **1B** show absolute and mean baseline tumor

Table 2: Baseline pO$_2$ (mmHg), maximum pO$_2$ (mmHg) and time to reach to maximum pO$_2$ (minutes) in different groups on day 1 to day 5 and day 10

Group	Time (days)					
	1	2	3	4	5	10
Baseline pO$_2$						
Group A	6.2±0.9	7.3±1.3	6.4±0.9	7.8±1.4	6.9±0.8	7.2±1.2
Group B	8.3±1.2	7.1±1.3	8.9±2.3	6.5±1.8	5.4±1.4	5.3±1.1
Groups C–F	6.6±0.8(C)		6.9±0.6(D)		5.4±0.4(E)	6.0±0.7(F)
Maximum pO$_2$						
Group A	19.2±4.0**	23.1±4.6*	14.0±1.8*	16.7±3.0	14.2±1.4**	14.1±2.4
Group B	29.4±8.2**	25.6±5.4**	19.7±5.0	22.7±9.4*	17.3±8.1*	5.0±1.4
Groups C–F	14.8±3.2*(C)		11.4±2.7*(D)		9.0±1.2*(E)	7.6±2.1(F)
Time to reach to maximum pO$_2$						
Group A	37.8±2.1	37.1±2.6	36.1±2.8	34.1±2.1	41.8±2.6	31.3±3.6
Group B	43.0±2.5	39.2±2.3	35.6±4.6	35.6±4.6	39.0±4.0	42.0±2.9
Groups C–F	41.2±3.4(C)		37.7±2.0(D)		38.3±2.8(E)	39.6±2.6(F)

Note: The baseline is the average of pO$_2$ from the 30-minute period before the inhalation of carbogen or 100% O$_2$. *$P < 0.05$, **$P < 0.01$, *vs.* the baseline on the same day (two-tailed paired *t*-test). pO$_2$: Partial pressure of oxygen.
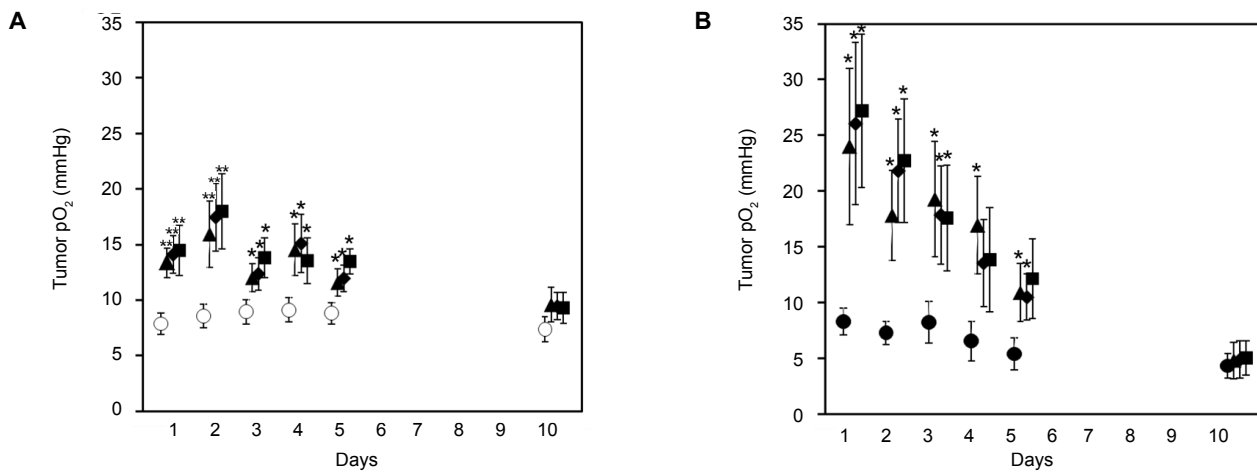

Figure 2: Average baseline tumor pO$_2$ and response to 60 minutes of carbogen (A) and 100% O$_2$ (B) inhalation.
Note: ○: baseline pO$_2$; ▲: 1–20 minutes; ♦: 21–40 minutes; ■: 41–60 minutes. *$P < 0.05$, **$P < 0.01$, *vs.* baseline pO$_2$ on the same day. pO$_2$: Partial pressure of oxygen.

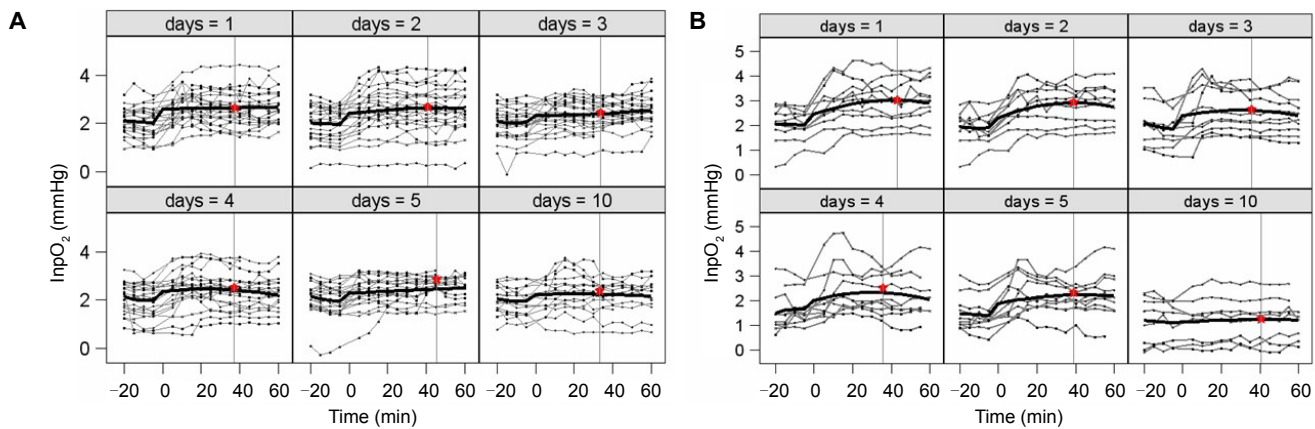**Figure 3: Time course of tumor pO$_2$ measured in individual mice prior to and during (A) carbogen and (B) 100% O$_2$ inhalation.**
Each line is pO$_2$ over time from an implant (2 implants/animal) from all animals ($N_{group A}$ = 9; $N_{group B}$ = 5), therefore, 16–18 lines for group A and 8–10 lines for group B per day. The bold line shows the average response of tumor pO$_2$ and the star * and the horizontal line shows the averaged maximum pO$_2$ (pO$_{2 max}$) and averaged time to reach to maximum pO$_2$ (T$_{max}$) for all animals in the group using an exponential quadratic function. pO$_2$: Partial pressure of oxygen; min: minute(s).

Table 3: Tumor volume (mm³) on days 1–5 and day 10 in different groups

Group	Time (days)					
	1	2	3	4	5	10
Group A	155±13	230±17	297±21	371±31	455±35	927±81
Group B	167±8	244±11	313±12	363±14	414±13	1 035±65
Groups C–F	164±6(C)		293±25(D)		458±9(E)	1 062±23(F)
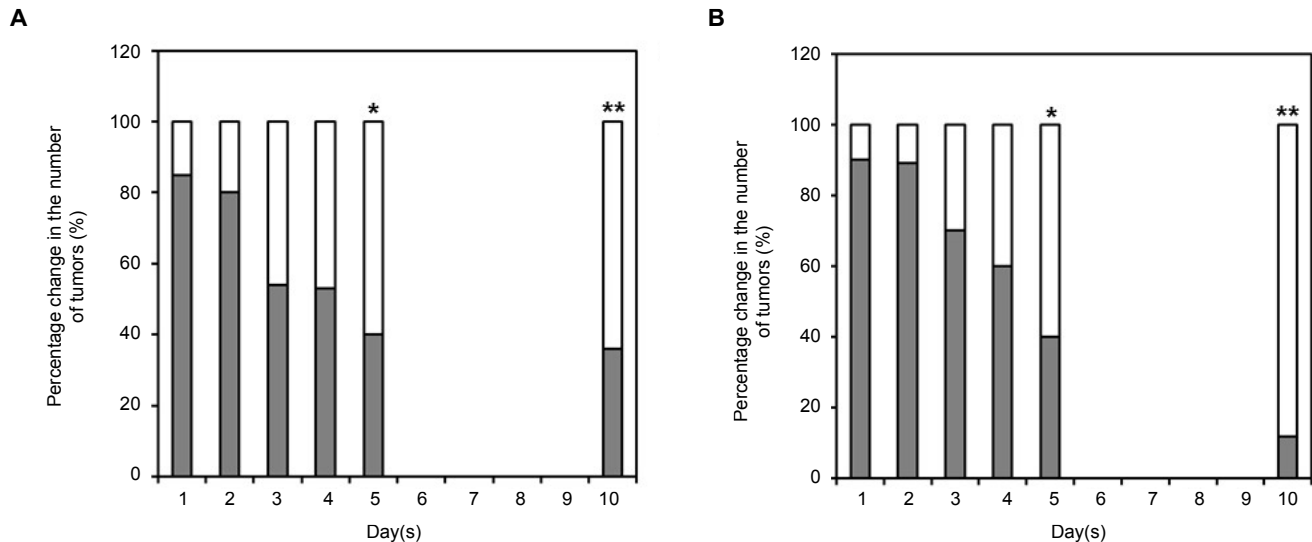
Figure 4: The percentage change in the number of tumors with pO₂ greater than 50% of the baseline (black column) and less than 50% of the baseline (white column) within the first 20 minutes of carbogen (A) and 100% O₂ (B) inhalation on each day.
Note: *$P < 0.05$, **$P < 0.01$, *vs*. day 1. pO₂: Partial pressure of oxygen.

pO₂ from individual LiPc implants on days 1 to 5 and day 10 in the groups A and B, respectively. The mean baseline pO₂ in groups A and B were $6.2 ± 0.9$ mmHg and $8.3 ± 1.2$ mmHg respectively on day1 (**Table 2**). The mean baseline tumor volumes on day 1 were $146 ± 10$ mm³ in group A and $167 ± 8$ mm³ in group B. No significant changes in the tumor pO₂ were observed by day 10; however the tumor volume increased significantly to $931 ± 64$ mm³ and $1,035 ± 65$ mm³ in group A and B, respectively (**Table 3**). There were no apparent effects of tumor volume on pO₂ (group A: $r = 0.24$; group B: $r = 0.14$).

The mean baseline pO₂ and an increase in pO₂ averaged over 20 minutes during 60 minutes of carbogen and 100% O₂ inhalations are shown in **Figure 2A** and **2B**, respectively. The mean tumor pO₂ during carbogen and 100% O₂ inhalation on days 1−5 were significantly greater than the baseline pO₂ ($P < 0.05$ or $P < 0.01$). The exponential quadratic function of time indicated a maximal increase in pO₂ at $34.1 ± 2.1$ to $41.8 ± 2.6$ minutes on day 1 and $35.6 ± 4.6$ to $43.0 ± 2.5$ minutes on day 5. However, no significant increase in tumor pO₂ on breathing carbogen or 100% O₂ was evident on day 10 (**Figure 3, Table 2**).

The tumors were categorized based on their increase in

pO₂ of more than 50% from the baseline within 20 minutes of breathing carbogen or 100% oxygen on each day, **Figure 4A** and **4B**, respectively. These results indicate a significant decrease in the percentage of tumors that responded to carbogen or 100% O₂ on day 5 ($P < 0.05$) and day 10 ($P < 0.01$) compared to day 1.

Effect of single administration of carbogen on tumor pO₂ and T_{max}

The absolute baseline tumor pO₂ from individual LiPc aggregates and the mean tumor pO₂ on day 1 (group C), day 3 (group D), day 5 (group E) or day 10 (group F) are shown in **Figure 5A**. The mean baseline pO₂ were $6.6 ± 0.8$ mmHg, $6.9 ± 0.6$ mmHg, $5.4 ± 0.4$ mmHg, $6.0 ± 0.7$ mmHg in groups C, D, E and F, respectively (**Table 2**). The mean tumor volumes as shown in **Table 3** were not significantly different than groups A and B on the corresponding days. The increase in the mean tumor pO₂ during carbogen inhalation was significantly higher than the baseline pO₂ ($P < 0.05$; **Figure 5B**). Maximal increase in tumor pO₂ was observed at $41.2 ± 3.4$ minutes on day 1, $37.7 ± 2.0$ minutes on day 3 and $36.3 ± 2.8$ minutes on day 5. However, no significant maximum increase in tumor pO₂ was observed on day 10
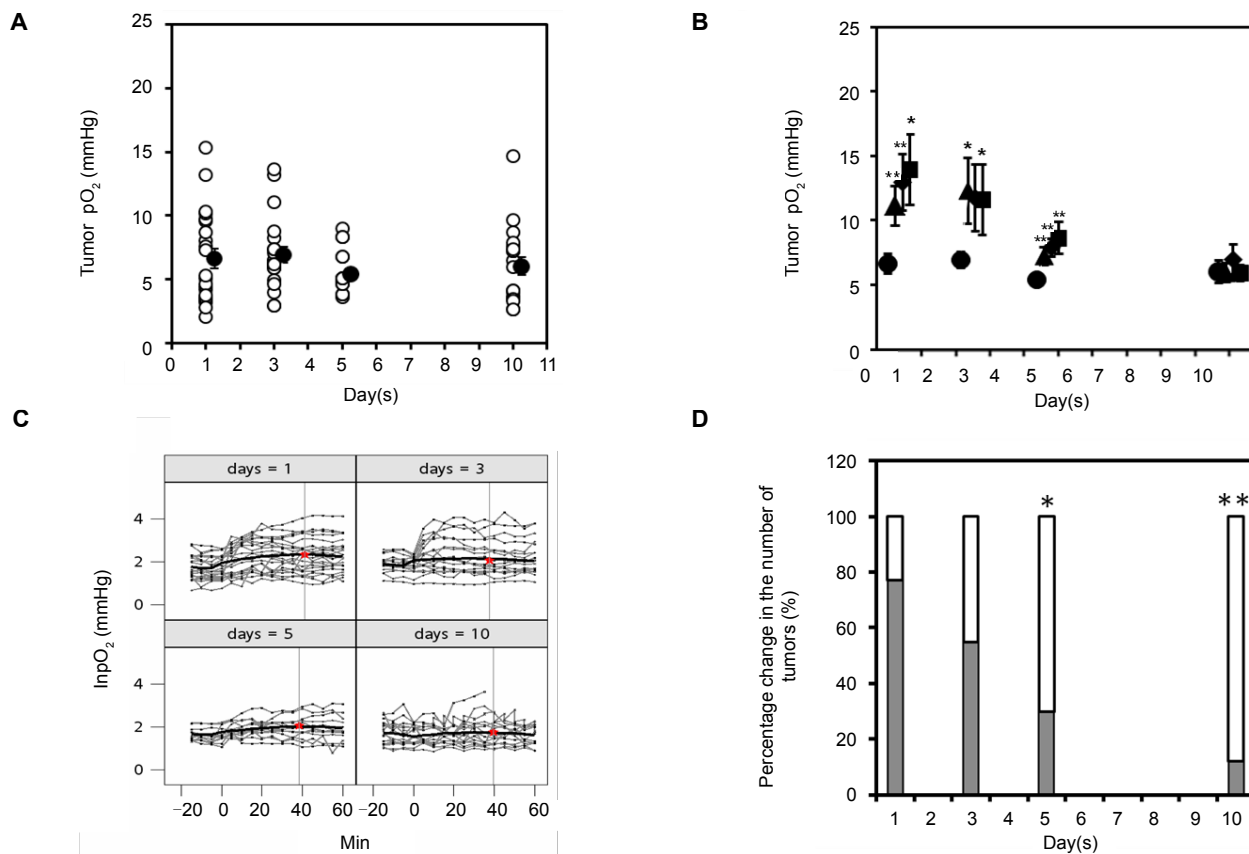
Figure 5: Effect of single administration of carbogen on tumor pO$_2$ and time to reach to maximum pO$_2$.

Note: (A) individual baseline pO$_2$ (○) recorded from each LiPc implant and mean pO$_2$ (●) in measurements repeated for 20 min on days 1, 3, 5 and 10. (B) Mean baseline tumor pO$_2$ (●) and increase in pO$_2$ at every 20 min of carbogen inhalation on day 1, 3, 5 and 10. (▲): 1–20 min carbogen inhalation; (♦): 21–40 min carbogen inhalation; (■): 41–60 min carbogen inhalation. *$P < 0.05$, **$P < 0.01$, $vs.$ baseline pO$_2$ in the same day. (C) Time course of tumor pO$_2$ measured in individual mice prior to and during carbogen inhalation on day 1, 3, 5 and 10. Each line is pO$_2$ over time from an implant (2 implants/animal) from all animals ($N_{day 1}$ = 10; $N_{day 3}$ = 11, $N_{day 5}$ = 6; $N_{day 10}$ = 10). The bold line shows the average response and the red stars * and the horizontal lines show the averaged maximum pO$_2$ (pO$_2$ max) and averaged time to reach to maximum pO$_2$ (T$_{max}$) for all animals in the group using an exponential quadratic function. (D) The percentage change in the number of tumors with pO$_2$ greater than 50% of the baseline (black column) and less than 50% of the baseline (white column) within the first 20 min of carbogen inhalation. *$P < 0.05$, **$P < 0.01$, $vs.$ day 1. pO$_2$: Partial pressure of oxygen; min: minute(s).

(**Figure 5C**, **Table 2**).

The percentage of tumors with an increase in pO$_2$ of more than 50% from the baseline within 20 minutes of breathing carbogen on days 1, 3, 5 and day 10 are shown in **Figure 5D**. The results indicate a significant decline in the tumors that responded to carbogen on day 5 ($P < 0.05$) and day 10 ($P < 0.01$) compared to day 1.

Effect of breathing carbogen or 100% O$_2$ on morphologic examination of the tumor

Gross and microscopic examination (hematoxylin-eosin staining) of the tissue confirmed that the LiPc aggregates were in the interstitial compartment of the tumor tissue with no evidence of edema or infiltration of inflammatory cells, although accumulation of blood cells and necrotic cells around the LiPc deposits was observed in some samples on day 5 (group E) and day 10 (group F) (**Figure 6**).

Discussion

The results reported here is the continuation of our systematic study to characterize the changes in tumor pO$_2$ during the inhalation of hyperoxic gases by EPR oximetry. We have previously reported the effect of breathing carbogen on the RIF-1, F98, C6 and 9L tumors pO$_2$ with the goal to improve oxygen levels for radiosensitization (Khan et al., 2009, 2010; Hou et al., 2011, 2012). The current results indicate that the RIF-1 tumors are hypoxic with a pO$_2$ of < 10 mmHg, consistent with earlier reports (Hasegawa et al., 1987; Hou et al., 2004, 2007, 2009, 2010, 2011, 2013). Kavanagh et al. (1996) reported a mean of 4.5 mmHg, when the diameter of RIF-1 tumor reached 11 ± 0.5 mmHg. With EPR oximetry, Bratasz et al. (2007) reported an average pO$_2$ of 4.9 mmHg in subcutaneous RIF-1 tumors of 113 mm^3 volume.

The temporal changes in tumor pO$_2$ with carbogen inhalation has varied in studies using different tumor types. Bus-
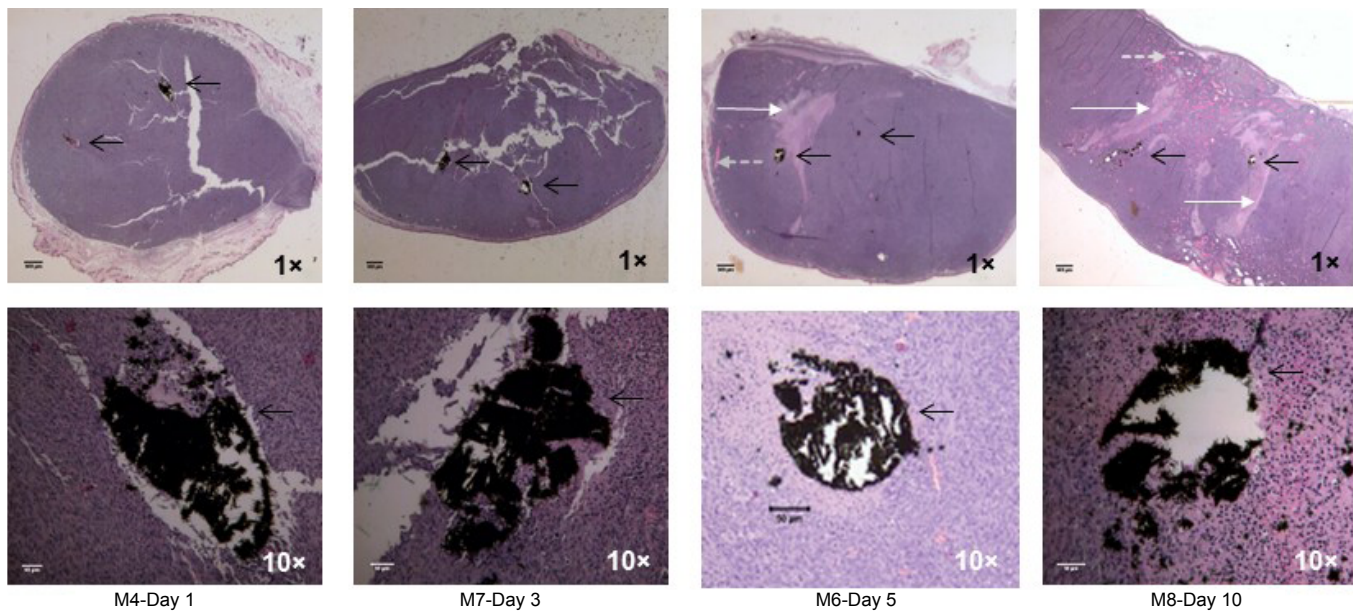
M4-Day 1   M7-Day 3   M6-Day 5   M8-Day 10

Figure 6: Effect of breathing carbogen on morphologic examination of the RIF-1 tumors (hematoxylin-eosin staining).
Note: The LiPc crystals (black color) are indicated by short black arrows, necrotic tissues are indicated by long white arrows and the blood cell accumulation is indicated by short white color dash line arrows. M4, M6, M7 and M8 represent the mouse numbers. The thickness of each section is 5 μm. Magnification: 1× (upper panel) and 10× (lower panel).

sink et al. (2000) reported a rapid increase in the tumor pO_2, with the mean T_{max} at approximately 3.9 (0.8−14) minutes and 3.6 (0.7−16) minutes in E102 and E106 glioblastoma xenografts, respectively. Gu et al. (2003) reported a significant increase in subcutaneous mammary adenocarcinoma tumors pO_2 within 8 minutes of carbogen breathing which gradually increased over the next 12 minutes. Thews et al. (2002) observed a slow increase in the pO_2 of subcutaneous rat DS-sarcomas over 15 minutes during carbogen challenge. We have observed a significant increase in the intracerebral F98 tumor pO_2 within 15 minutes of carbogen inhalation (Hou et al., 2012). On the other hand, a significant increase in the RIF-1 tumor pO_2, with a T_{max} at 22.9−31.2 minutes on days 1−5 post carbogen inhalation was observed (Hou et al., 2011). In the present study, carbogen inhalation significantly increased tumor pO_2, with a maximum increase at 32−44 minutes on days 1−5. These results indicate that the time to achieve a maximal increase in tumor pO_2 with carbogen inhalation depends on the tumor type, tumor location, and possibly tumor size. A significant decline in tumor pO_2 during repeated breathing of 100% oxygen inhalation over days was also observed. Similar results has been observed previously in RIF-1 (Hou et al., 2011) and also 9L, C6 tumor models (Khan et al., 2009). This is likely due to a combination of several factors, such as an increase in interstitial pressure, compromised tumor vasculature with increase in tumor size and increase in necrotic areas in the tumor over days (Khan et al., 2009).

Several investigators have used percentage pO_2 values

≤ 5 mmHg as a measure of a clinically significant hypoxic fraction. Fyles et al. (1998) presented the hypoxic proportion, defined as the percentage of pO_2 values less than the median value of 5 mmHg in patients with cervix cancer and found grouping of patients above and below the percentage of pO_2 readings of < 5 mmHg of 50% (closely approximating the median value of 52%) resulted in a 2.9-fold greater risk of failure for hypoxic tumors. A group of patients with bulky hypoxic tumors appears to have a substantially higher risk of tumor recurrence or death tumors with > 50% change in pO_2 from pretreatment had a poor outcome. We have observed a significant decline in the percentage of tumors with an increase in pO_2 of more than 50% from the baseline on day 5 and day 10 compared to day 1 during multiple or single administration of carbogen. These results indicate that the response to carbogen is not consistent over days and therefore likely to compromise the efficacy of fractionated radiotherapy, which is usually administered for 4−6 weeks. On the other hand, fractionated radiotherapy can also alter the response of tumors to carbogen due to cell kill and effect on the tumor vasculature (Song et al., 1987; Yaromina et al., 2011; Multhoff and Vaupel, 2012). This will be the focus of our future study in the quest to effectively use carbogen to oxygenate and radiosensitize the tumors. The histological results indicate some accumulation of necrotic cells around the LiPc aggregates in some samples on day 5 (group E) and day 10 (group F). These were not caused by the LiPc implants, rather reflects the normal histological pattern of the tumor. These results are consistent with our previous

report on the histological appearance of solid tumors (Hou et al., 2013).

In conclusion, these results provide quantitative information on the effect of carbogen and 100% oxygen inhalation in enhancing tumor oxygenation over the course of 10 days. Since the time to achieve a significant increase in tumor pO_2 is likely to vary with the tumor type, size, and site, EPR oximetry can be used to repeatedly monitor tumor pO_2 to test hyperoxic interventions and confirm tumor oxygenation.

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