Noninvasive measurement of microvascular and interstitial oxygen profiles in a human tumor in SCID mice

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ABSTRACT Simultaneous measurements of intravascular and interstitial oxygen partial pressure (Po2) in any tissue have not previously been reported, despite the importance of oxygen in health and in disease. This is due to the limitations of current techniques, both invasive and noninvasive. We have optically measured microscopic profiles of Po2 with high spatial resolution in subcutaneous tissue and transplanted tumors in mice by combining an oxygen-dependent phosphorescence quenching method and a transparent tissue preparation. The strengths of our approach include the ability to follow Po2 in the same location for several weeks and to relate these measurements to local blood flow and vascular architecture. Our results show that (i) Po2 values in blood vessels in well-vascularized regions of a human colon adenocarcinoma xenograft are comparable to those in surrounding arterioles and venules, (ii) carbogen (95% O2/5% CO2) breathing increases microvascular Po2 in tumors, and (iii) in unanesthetized and anesthetized mice Po2 drops to hypoxic values at <200 μm from isolated vessels but drops by <5 mmHg (1 mmHg = 133 Pa) in highly vascularized tumor regions. Our method should permit noninvasive evaluations of oxygen-modifying agents and offer further mechanistic information about tumor pathophysiology in tissue preparations where the surface of the tissue can be observed.

Oxygen is an important determinant of tumor growth and response to various therapies—e.g., radiation therapy, chemotherapy, and photodynamic therapy (1–3). This key role of oxygen has led to numerous theoretical and experimental investigations of oxygen partial pressure (PO2) in rodent and human tumors. Most data on tissue PO2 have been obtained by direct measurements using oxygen-sensing electrodes (1–12). Although such measurements are useful, there are several problems associated with them. (i) Electrodes are invasive and therefore may require the use of anesthetics. (ii) PO2 is measured only along the electrode track, thus limiting the volume of tissue sampled. (iii) Repeated measurements in the same location in an unperturbed state over a long time period (several weeks) to study the effect of modifiers of oxygenation are not possible. (iv) The distances between the electrode tip and nearby blood vessels are not generally known. Indirect methods based on the detection of an oxygen-sensitive chemical probe by nuclear magnetic resonance, positron emission tomography, or electron paramagnetic resonance may overcome some of these problems, but these noninvasive methods do not have the spatial resolution to measure microvascular and interstitial PO2 gradients (13–16). Quantitative autoradiography of tissues after injection of hypoxia-sensitive probes can provide the required spatial resolution, but this method is invasive and hence does not permit longitudinal studies in the same tumor.

Wilson and coworkers (17–19) recently pioneered a phosphorescence technique and obtained macroscopic, two-dimensional images (12,000 μm x 8000 μm) of PO2 in normal and tumor tissue of anesthetized animals. We have used this technique to obtain PO2 values in single microvessels and the interstitial PO2 distribution at known distances from blood vessels with higher spatial resolution (~15 μm). The PO2 value decreases with distance from a capillary due to diffusion and/or perfusion limitation for a fixed rate of oxygen consumption. The only tumor measurements in the literature where this distance is known are due to Dewhirst et al. (20). However, their microelectrode technique was invasive, and their tumor preparation required superfusing the tissue. Moreover, it did not permit simultaneous intravascular PO2 measurements.

We report here a noninvasive microscopic approach based on a modified Algire chamber preparation in severe combined immunodeficient (SCID) mice (21) and oxygen-dependent phosphorescence quenching (17–19, 43) to measure PO2 in the microvascular and interstitial space of a human tumor xenograft. Blood flow and PO2 were measured with and without anesthesia in animals breathing air or carbogen.

MATERIALS AND METHODS

Tumor Preparation. Dorsal skin chambers were implanted in SCID mice (Fig. 1) (21). This preparation allows one to monitor continuously and noninvasively microvessels and interstitium of subcutaneous skin tissue and transplanted tumors for up to 1 month. It also allows the simultaneous measurements of blood flow and PO2 in microvessels as small as capillaries. Individual microcirculatory measurements were performed in five normal and six tumor-bearing chambers. Human colon adenocarcinoma LS174T was grown in the chamber for 14–18 days, until it reached a diameter of 4–6 mm.

In Vivo Microscopy. Observations of the microcirculation were performed under an inverted microscope (IMT-2, Olympus, New Hyde Park, NY) using a ×20 objective and brightfield transillumination (halogen lamp, 12 V, 100 W). Images detected by a television camera (model 4815, Cohu, San Diego) were displayed on a television monitor (PVM-1342Q, Trinitron; Sony, Tokyo) and recorded on a video cassette recorder (AG-500; Panasonic, Secaucus, NJ). Analysis of erythrocyte velocities and vessel diameters was performed off-line by means of a modified four-slit videophotometric technique (Microflow System, model 208C, video photometer version; IPM, San Diego) and an image shearing monitor (digital image shearing monitor, model 908; IPM). The blood flow rate was calculated as described by Leung et al. (21). We did not classify tumor vessels as arteriolar or venular, since such a classification is not applicable to tumors (22, 23).

Oxygen Measurement. For PO2 measurements, albumin-bound palladium (Pd)-coproporphyrin compound (Porphyrin Products, Logan, UT) was injected (13 mg/ml; 0.05 ml intravenously every 2 hr) into the bloodstream of the animal. Binding of the Pd-coproporphyrin to albumin increases its

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plasma half-life. Since blood vessels of tumor periphery are leaky (24–27), this compound readily extravasates into the interstitium and reaches uniform concentrations in <15 min, thus permitting vascular and interstitial \( P_{o_2} \) measurements. The tissue region of interest was excited with a flashlamp (decay constant, 10 \( \mu \)sec; frequency, 30 Hz; peak wavelength, 420 nm) (EG & G, Salem, MA) and phosphorescence signals from the superficial tissue layer were detected (630-nm cutoff filter). Depth of phosphorescence signal collection throughout the tissue was \( \approx 10 \mu \)m for the \( \times 20 \) dry objective (\( \times 20 \), SPlan, Olympus, Tokyo) used for all measurements, as determined \( \text{in vivo} \) by focusing and defocusing the region of interest. Only in exactly focused areas could phosphorescence signals be detected by the photomultiplier. The measuring “window” was 100 \( \mu \)m parallel and 15 \( \mu \)m perpendicular to the vessel wall. Both the lifetime (\( \tau \) and intensity of the emitted phosphorescence are dependent on oxygen concentration and can be used to measure \( P_{o_2} \); however, lifetime measurements are superior because they are independent of the local probe concentration and light intensity. The decay of the emitted phosphorescence following the excitation was monitored as a function of time by means of a photomultiplier tube (model 9855B, Thorn EMI Electron Tubes, Rockaway, NJ), digitized by an oscilloscope (model 2434, Tektronix), and stored on a microcomputer (43).

The phosphorescence lifetime (\( \tau \)) was calculated and converted into \( P_{o_2} \) according to the Stern–Volmer equation:

\[
P_{o_2} = \frac{1}{k} \left( \frac{1}{\tau} - \frac{1}{\tau_0} \right),
\]

where \( k \) is the quenching constant and \( \tau_0 \) is the value of \( \tau \) at zero \( P_{o_2} \). Both constants (\( \tau_0 \) and \( k \)) are dependent on pH and temperature, but for the albumin-bound Pd-coproporphyrin the pH dependence is negligible in the range 6.8–7.6 as

![Fig. 1.](image)

*(Upper) Dorsal skin-fold chamber in SCID mice was used to visualize microvessels in the subcutaneous tissue and in human colon adenocarcinoma LS174T transplanted into this preparation. The titanium chamber sandwiches a double layer of extended dorsal skin containing the microcirculatory preparation. (Lower) Transillumination photograph of an LS174T tumor (diameter, 5 mm) localized in the center of the chamber preparation, 14 days after tumor-cell implantation.*

<table>
<thead>
<tr>
<th>Vessel Condition</th>
<th>( P_{o_2} ), mmHg</th>
<th>( V ), mm/sec</th>
<th>( D ), ( \mu )m</th>
<th>( Q ), ( 10^{-6} ) ml/sec</th>
<th>Linear correlation, ( r^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumor vessels</strong></td>
<td><strong>(N = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ua</strong></td>
<td>29.9 ± 11.3 (27)</td>
<td>0.21 ± 0.09 (24)</td>
<td>37.8 ± 29.5 (27)</td>
<td>133.2 ± 171.8 (24)</td>
<td>0.068</td>
</tr>
<tr>
<td><strong>Uc</strong></td>
<td>45.1 ± 21.7 (27)</td>
<td>0.22 ± 0.12 (25)</td>
<td>36.5 ± 29.3 (27)</td>
<td>131.7 ± 178.1 (25)</td>
<td>0.294</td>
</tr>
<tr>
<td><strong>Aa</strong></td>
<td>16.4 ± 7.8 (27)</td>
<td>0.21 ± 0.16 (25)</td>
<td>32.0 ± 27.6 (27)</td>
<td>95.4 ± 137.9 (25)</td>
<td>0.508</td>
</tr>
<tr>
<td><strong>Ac</strong></td>
<td>40.6 ± 21.8 (27)</td>
<td>0.19 ± 0.13 (24)</td>
<td>32.7 ± 26.8 (27)</td>
<td>108.8 ± 158.4 (24)</td>
<td>0.675</td>
</tr>
<tr>
<td><strong>Arterioles</strong></td>
<td><strong>(N = 5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ua</strong></td>
<td>26.4 ± 9.5 (14)</td>
<td>(&gt;1.2)</td>
<td>34.7 ± 30.0 (14)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Uc</strong></td>
<td>49.5 ± 22.4 (12)</td>
<td>(&gt;1.2)</td>
<td>34.4 ± 31.5 (14)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Aa</strong></td>
<td>17.7 ± 6.1 (14)</td>
<td>(&gt;1.2)</td>
<td>34.4 ± 29.6 (14)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Ac</strong></td>
<td>31.0 ± 18.5 (14)</td>
<td>(&gt;1.2)</td>
<td>32.7 ± 30.1 (11)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Venules</strong></td>
<td><strong>(N = 5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ua</strong></td>
<td>25.3 ± 5.6 (43)</td>
<td>0.30 ± 0.13 (35)</td>
<td>38.4 ± 24.7 (43)</td>
<td>319.7 ± 428.5 (35)</td>
<td>0.083</td>
</tr>
<tr>
<td><strong>Uc</strong></td>
<td>36.0 ± 10.9 (42)</td>
<td>0.28 ± 0.09 (35)</td>
<td>38.7 ± 22.5 (43)</td>
<td>281.8 ± 468.5 (35)</td>
<td>-0.081</td>
</tr>
<tr>
<td><strong>Aa</strong></td>
<td>15.7 ± 4.2 (40)</td>
<td>0.29 ± 0.11 (35)</td>
<td>36.3 ± 21.6 (39)</td>
<td>299.9 ± 544.3 (35)</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>Ac</strong></td>
<td>20.6 ± 10.5 (40)</td>
<td>0.25 ± 0.10 (36)</td>
<td>37.4 ± 22.0 (40)</td>
<td>233.0 ± 330.1 (36)</td>
<td>0.206</td>
</tr>
</tbody>
</table>

\( N \), no. of animals; **Ua**, unanesthetized, air breathing; **Uc**, unanesthetized, carbon breathing; **Aa**, anesthetized, air breathing; **Ac**, anesthetized, carbon breathing. Values are mean ± SD with no. of measured vessels (\( n \)) and range of values in parentheses. Erythrocyte velocity of arterioles is >1.2 mm/sec and cannot be measured by our system. Thus, blood flow rate could not be calculated for arterioles.

*Pearson correlation coefficient.

*Fig. 1.*
determined \textit{in vitro}. At 32°C and pH 7.0, the \textit{in vitro} calibration gives $k = 240 \text{ mmHg}^{-1}\text{sec}^{-1}$ and $\tau_0 = 940 \mu\text{sec}$, respectively (1 mmHg = 133 Pa). Since the values of $\tau_0$ and $k$ are specific to the compound bound to albumin at a given pH and temperature, $\tau_0$ and $k$ determined \textit{in vitro} hold for measurements \textit{in vivo} (17–19). The uncertainty (\Delta Po$_2$) of calculated Po$_2$ values depends on the errors in $\tau_0$ and $k$,

$$\Delta \text{Po}_2 = \text{Po}_2 \frac{\Delta k}{k} + \frac{\Delta \tau_0}{\tau_0}.$$ 

In our study, the maximum value of $\Delta k/k$ or $\Delta \tau_0/\tau_0$ was <4%. Although the method provides sensitivity to oxygen over the whole physiological Po$_2$ range, the determinations are particularly accurate at low Po$_2$. For instance, in the range 0–5 mmHg, a ±15% error in the estimation of $\tau$ leads to a calculated Po$_2$ of less than ±1 mmHg, whereas at the level of 30 mmHg, the calculated Po$_2$ would change ±5 mmHg for the same variability in the estimation of $\tau$.

**Experimental Protocol.** Erythrocyte velocity, vessel diameter, and Po$_2$ were measured in six tumor-bearing and five control animals. In each animal, at least eight intravascular or extravascular regions were studied. Each animal was studied both in the awake (U) and in the anesthetized condition (A; 7.5 mg of ketamine hydrochloride and 2.5 mg of xylazine per 100 g of body weight, subcutaneously), breathing either room air (a) or carbogen (c; 95% O$_2$/5% CO$_2$). The time sequence of the four different combinations of treatment (Ua, Aa, Uc, Ac) for each animal was randomly selected and the same region was studied under each treatment. Taken together, >100 different regions were studied, and >400 measurements were performed for each parameter. In spite of the variability of individual responses, the results were qualitatively similar for each animal studied. The temperature of the skin in the area under study was periodically measured with a thermocouple (Physitemp, Clifton, NJ). Individual skin temperatures were then used to calculate the appropriate values of $\tau_0$ and $k$ for every animal under each experimental situation.

Data groups were statistically compared by the least-mean-square method, and linear correlations were tested with the Pearson correlation coefficient.

**RESULTS AND DISCUSSION**

Due to the invasive nature of the currently used approaches, almost all Po$_2$ data have been obtained in anesthetized animals. Our approach enabled us to compare blood flow and Po$_2$ in awake and in anesthetized animals. Table 1 shows Po$_2$, erythrocyte velocity, vessel internal diameter, and blood flow rate in individual vessels in awake and anesthetized animals. Table 2 gives the significance levels for Po$_2$ values under these conditions. Note that anesthesia did not significantly alter vessel diameter, erythrocyte velocity, or blood flow rate despite an ~20% reduction in mean arterial pressure and an ~35% decrease in heart rate in these mice (21). Intravascular Po$_2$, however, significantly decreased in normal arterioles and venules and in tumor vessels, probably due to a reduction (22%) in breathing rate (Fig. 2). Our Po$_2$ values in arterioles are comparable to reported values (28–31).

Various studies in the literature have suggested that human tumors have a higher Po$_2$ and/or hemoglobin oxygen saturation compared with rodent tumors (1–16). Our results on the effect of anesthesia on Po$_2$ offer a possible explanation for these differences. Most human Po$_2$ studies were conducted in unanesthetized patients, whereas animal studies were usually done under anesthesia. These differences may become even larger if anesthesia decreases the tumor blood flow rate in addition to suppressing breathing rate.

We did not find a statistically significant difference in Po$_2$ between normal and tumor vessels (Table 1). This does not exclude the possibility that the blood vessels in the deeper region of our tumor model may be less oxygenated than in the periphery. However, our method permits measurements only at the tumor surface. This differs from results of Wilson and Cerniglia (17), who reported that 9L glioma vessels have a lower Po$_2$ than that in the surrounding normal tissue in anesthetized animals. In their study, macroscopic phosphorescence measurements in tumors contained signals from intravascular and interstitial spaces due to "leaky" structure.

![Fig. 2. Mean and standard deviation of relative Po$_2$ values of three (sequential) measurements in the venules (n = 5, n = 40) (open bars) and arterioles (n = 5, n = 12) (filled bars) of the subcutaneous tissue of the dorsal skin-fold chamber and in blood vessels (N = 6, n = 27) of the LS174T human adenocarcinoma (hatched bars) implanted into the dorsal skin chamber preparation (mean vessel diameter, 35 μm). The Po$_2$ level of the awake animal breathing air was taken as the baseline (see Table 1). Under these experimental conditions no significant differences of Po$_2$ values between subcutaneous tissue venules and arterioles and the tumor blood vessels were found. In all vessels, carbogen breathing increased Po$_2$ in unanesthetized mice. Anesthesia in animals breathing normal room air decreased local Po$_2$, whereas carbogen breathing of anesthetized mice again increased Po$_2$ in venules, arterioles, and tumor blood vessels. (For statistical significance, see Table 2.)](image-url)

**Table 2.** Significance levels for Po$_2$ values as tested by the least-mean-square method.

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Ua</th>
<th>Aa</th>
<th>Ac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>Ua</td>
<td>0.0091</td>
<td>0.1843</td>
</tr>
<tr>
<td></td>
<td>Aa</td>
<td>0.0002</td>
<td>0.1375</td>
</tr>
<tr>
<td>Arterioles</td>
<td>Ua</td>
<td>0.0004</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Aa</td>
<td>0.0001</td>
<td>0.1909</td>
</tr>
<tr>
<td>Venules</td>
<td>Ua</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Aa</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Ua, unanesthetized, air breathing; Uc, unanesthetized, carbogen breathing; Aa, anesthetized, air breathing; Ac, anesthetized, carbogen breathing.
of tumor vessels (see below) whereas normal tissue measurements were based on intravascular signals. Since interstitial 
Po2 is less than microvascular Po2, it is likely that those tumor 
Po2 values were a weighted average of intra- and extravascul
ar values.

Most preparations used in microvascular research rely on 
the direct visualization of the microvessels, and the technique should be applicable in most circumstances. Our system extends the range of applicability of the phosphorescence technique and allowed Po2 determinations in areas at least 3 orders of magnitude smaller than previously reported. In addition, the system allowed measurement of other microcirculatory parameters, such as microvessel diameter and blood flow, at the same location (43). The contribution from areas below the vessel under study was probably small because the emission from those regions is strongly absorbed by the intravascular blood column.

Several agents have been used to increase tumor Po2 to enhance the outcome of radiation therapy (2,3). One method is to use carbogen breathing (32). As shown in Fig. 2, carbogen breathing increased vascular Po2 in both unanesthetized and anesthetized mice.

Leakage of the albumin-bound porphyrin out of the blood pool in vessels at the tumor periphery (24-27) enabled us additionally to measure Po2 in interstitial regions of LS174T tumors. We measured Po2 profiles around single vessels up to a distance of ~350 μm from the vessel wall in poorly vascularized regions and between two parallel vessels, ~100 μm apart in well-vascularized regions, with a resolution of 15 μm perpendicular to the blood vessel. A total of 11 gradients were determined, all showing the same trend. Figs. 3 and 4 present examples of individual Po2 profiles. Note that the interstitial Po2 values are smaller than and depend strongly on the intravascular Po2 of the nearby blood vessels. Large variations among intravascular Po2 in tumors led to large differences in interstitial Po2. In our chamber preparation, vessels are accessible only at the tumor surface, where vessel density is highest. In the rare cases when we found interves-
sel distances of >200 μm, Po2 dropped to hypoxic values at 
~200 μm, which corresponds to observations and calculations previously reported (33-39). On the other hand, the slight Po2 drop between two parallel vessels <100 μm apart was less than that calculated from a single isolated Krogh cylinder analysis (33-39). However, when one considers the interaction between vessels, this slight drop is not surprising (33-39).

In conclusion, we have presented intravascular and inter-
stitial measurements of Po2. Although our approach has certain limitations and cannot replace invasive as well as noninvasive methods currently used in the clinic, it will permit the testing of hypotheses on tissue oxygenation and the evaluation of oxygen-modifying agents, and it will offer further mechanistic information about tumor pathophysi-
ology (40-42).

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