Determination of Local Oxygen Consumption Rates in Tumors

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Abstract

At any location in a respiring tissue, partial pressure of oxygen (P02) is influenced by the local oxygen consumption rate. Consumption rates in vascular tumor tissues have previously been estimated for macroscopic regions. Using oxygen electrodes, we measured P02 profiles across microregions (87 μm to 286 μm wide) of tumors (R3230AC mammary adenocarcinoma) in a rat dorsal skin flap preparation and mapped adjacent microvessels. By comparing measured P02 values with theoretical simulations, we deduced local consumption rates. Results for six profiles ranged from 0.83 to 2.22 cm3 O2/100 g/min. The mean (±SD) was 1.52 ± 0.51 cm3 O2/100 g/min. This technique permits investigation of variations in consumption at a microscopic level.

Introduction

Partial pressure of oxygen (P02) of tumor tissue strongly influences its metabolic state and its response to radiation and other treatments. At each point in the tissue, P02 depends on the supply of oxygen by the microvessels and on the local metabolic demand. The rate of oxygen consumption is thus an important factor in tissue oxygenation. Numerous measurements have been made of oxygen consumption by macroscopic regions of tumors, yielding values over a wide range up to about 4 cm3 O2/100 g/min (1). However, determinations of local oxygen consumption on microscopic scales (of order 100 μm) have not been reported previously for vascular tissues, including tumors.

Typical diffusion distances for oxygen in tumors are in the range 40 μm to 200 μm (2, 3), and P02 at any location, therefore, depends on the consumption rate within a surrounding region with a radius in this range. Inhomogeneities of tissue metabolism and of oxygen availability can cause variations of consumption; therefore, local consumption does not necessarily equal average consumption over a macroscopic region. Estimates of local oxygen consumption are necessary in order to obtain a detailed understanding of the balance between oxygen supply and demand at the microscopic level.

The supply of oxygen depends on the flow and oxygen content of blood perfusing the tissue and on the geometric arrangement of the microvessels responsible for oxygen exchange (4, 5). Theoretical simulations (3) show that the heterogeneous structure of tumor microcirculation strongly influences the distribution of P02 and the occurrence of hypoxic microregions. These results emphasize both the importance of the local oxygen consumption rate in understanding tumor oxygenation and the likelihood that consumption is significantly nonuniform.

Here, we report determinations of oxygen consumption rate in microscopic tumor tissue regions bounded by microvessels. Profiles of tissue P02 were measured along lines crossing the regions using oxygen microelectrodes. Theoretical simulations of oxygen diffusion in the regions were made, and consumption rates were estimated for best fit between measured and simulated values. This theoretical method is potentially useful also for estimating oxygen consumption from profiles of tissue P02 measured by quench phosphorescence (6).

Methods and Materials

Animal Model. Fischer 344 rats weighing 125—150 g (Charles River, Raleigh, NC) were used for implantation of transparent window chambers as described previously (7). A 0.1-mm2 piece of the R3230AC mammary adenocarcinoma was transplanted into the window at the time of surgery. The animals were kept in a controlled environment of 35°C/50% relative humidity until the time of experimentation. Measurements were performed 9—10 days post surgery. The tumors were vascularized and averaged 2—3 mm in diameter at this time.

Microelectrodes. Whalen-type microelectrodes were manufactured and calibrated in our laboratory as described previously (8). These electrodes have a tip diameter of 3—6 μm with a recessed, gold-plated sensing surface. All electrodes were calibrated at least three times in saline at 33°C (the temperature of the tissue in the window chamber at the time of experimentation) with certified gas mixtures of 0, 2.5, 5, 15, and 21% O2 in N2 in a grounded, shielded enclosure. Electrodes that consistently gave linear (r2 > 0.85) calibration curves with a sensitivity of >0.2 pA/mM Hg were used.

Intravital Video Microscopy. Tumor microvasculature was visualized by transillumination (Zeiss Photomicroscope III; equipped with 6.3× and 20× objectives). The images were videotaped (Hamamatsu 2400; Hamamatsu, Hamamatsu City, Japan) onto SVHS videotape (Mitsubishi BV-100; Mitsubishi Electronics, Japan). The magnification factors of the images on the video monitor were 500× for the 20× objective and 150× for the 6.3× objective. A video time signal was superimposed on the video images for recording purposes (Model VTG-55; For A. Co., Ltd., Los Angeles, CA).

Experimental Protocol. The animals were anesthetized ip with 40 mg/kg Nembutal. A femoral artery was fitted with a catheter for measurement of mean arterial pressure (Fiberoptic Sensor Technologies, Inc., Ann Arbor, MI). The animal was placed on the microscope stage in lateral recumbency on a thermostatically controlled heating blanket (Harvard homeothermic blanket; Harvard Apparatus, Ltd., South Natick, MA). The upper window was removed from the window chamber, and the surface of the tissue within the chamber was suffused with Earle’s balanced salt solution, bubbled with 100% N2, and heated in a water-jacketed chamber such that the temperature of the medium reaching the chamber surface was 33°C. We have previously shown that this suffusion method creates an average P02 measurement on the tissue surface of 4 mmHg (8).

The objective of the protocol was to measure a profile of oxygen tension between two vessel segments in order to provide data for calculation of oxygen consumption rates. The method relies on the identification of an isolated one- or two-vessel network where vascular segments are roughly parallel over a distance of >50 μm. Here, a one-vessel network consists of a vascular loop. Networks of this type had to be free of out-of-focal-plane vessels that were closer than 100 μm to the network of interest. In addition, the vessels had to be at least 50 μm beneath the tissue surface in order to avoid artifacts that could be created by the suffusion medium (8, 9). Once a network fulfilling these criteria was found, measurements were performed at the wall of each vessel segment and at several interstitial points between the two segments in a track that was normal to the orientation of the segments (Fig. 1). Distances between measured points were also determined.

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LOCAL OXYGEN CONSUMPTION RATES IN TUMORS

Theory. Outside of the blood vessels, oxygen transport occurs by diffusion and is described by:

\[ Dc \left( \frac{\partial^2 P}{\partial x^2} + \frac{\partial^2 P}{\partial y^2} + \frac{\partial^2 P}{\partial z^2} \right) = M \]  

(A)

where \( D \) and \( c \) are, respectively, oxygen diffusivity and solubility in tissue; \( P \) is \( P_{O_2} \); \( x \), \( y \), and \( z \) are Cartesian coordinates; and \( M \) is the consumption rate. We assume that \( M \), \( D \), and \( c \) are uniform within the region and that the Krogh diffusion coefficient \( Dc = 4.2 \times 10^{-10} \text{ cm}^3 \text{ O}_2 \text{ per cm/s/mmHg} \) (2). If consumption rate varies within the region, \( M \) represents a mean value. At the blood-tissue boundary, \( P_{O_2} \) and oxygen fluxes must be continuous. Appropriate boundary conditions are also required at the outer boundaries of the tissue domain, as discussed below.

Fully three-dimensional solutions of Eq. (A) can be computed for specific configurations of vessels and tissue (3). Such computations are relatively complex and require the specification of many parameters, some of which are difficult to estimate. Therefore, we first consider simpler one- and two-dimensional solutions of Eq. (A), as indicated in Fig. 2.

For one-dimensional solutions, we assume that oxygen diffusion occurs solely in the direction parallel to the measured profile (Fig. 2a). Then, tissue \( P_{O_2} \) is governed by:

\[ Dc \frac{\partial^2 P}{\partial x^2} = M \]  

(B)

where \( x \) is distance along the profile, and other variables are as above. This yields:

\[ P = a + bx + cx^2 \]  

(C)

where \( a \) and \( b \) are constants that depend on the boundary conditions. This solution neglects diffusion of oxygen into tissue above or below the plane of the vessels. Such diffusion can be included by considering a two-dimensional equation:

\[ Dc \left( \frac{\partial^2 P}{\partial x^2} + \frac{\partial^2 P}{\partial z^2} \right) = M \]  

(D)

Here, \( x \) is the distance along the measured profile and \( z \) represents the depth in the tissue. Vessels are assumed to supply oxygen to a region of finite thickness and to lie in the midplane of this region (Fig. 2b). The thickness of the tissue and depth of the vessel locations within the tissue were not measured. We performed simulations for tissue thicknesses of 100 and 200 \( \mu \text{m} \), since the actual thickness is in this range. A number of standard methods are available to solve the problem of diffusion from vessels of circular cross-section embedded in a rectangular domain. We used a two-dimensional version of the Green’s function method (see below).

In both the one- and two-dimensional simulations, \( P_{O_2} \) at each point depends linearly on three unknown parameters, \( P_{O_2}S \) at the two vessel segments bounding the region and the consumption rate. For each region studied, the dependence of the predicted \( P_{O_2} \) at each measurement location on these three parameters was determined. The values of the parameters were then estimated for each region by minimizing the mean square deviation between simulated and measured values of \( P_{O_2} \) at the measurement locations. The correlation coefficient \((r^2)\) of measured and predicted \( P_{O_2} \) was also computed.

The two-dimensional simulation neglects diffusion resulting from gradients in \( P_{O_2} \) in the direction parallel to the vessels. To test the effects of such diffusion on simulated profiles, we carried out three-dimensional simulations using the Green’s function method for one of the configurations (Fig. 2c). This method has been described in detail (3, 10). Briefly, the microvessels in the region are subdivided into a finite number of short segments, each representing a source (or sink) of oxygen, of unknown strength. The \( P_{O_2} \) field in the tissue is represented as a superposition of the \( P_{O_2} \) fields resulting from these short segments, and the condition of continuity of \( P_{O_2} \) between blood and tissue is imposed, allowing calculation of the source strengths.

Results and Discussion

Eight complete \( P_{O_2} \) profiles were obtained. One was excluded from the analysis for two reasons; most of the tissue between the vessels was hypoxic, and it could not be fitted satisfactorily by the one- or two-dimensional simulations. Fig. 3 shows the measured \( P_{O_2} \) values for the remaining six cases, together with the best-fit profiles derived from one- and two-dimensional simulations, whereas Table 1 lists the corresponding estimates of consumption and the correlation coefficients between measured and simulated values. In the case of the two-dimensional simulations, the results shown are for a tissue thickness of 100 \( \mu \text{m} \).

Both one- and two-dimensional simulations led to predicted \( P_{O_2}S \) that correlated reasonably well with measured values, with \( r^2 \)s ranging from 0.55 to 0.98. The simulations cannot, however, account for local maxima in measured \( P_{O_2} \) within the region, which presumably result either from the presence of other microvessels that were not visualized or from random measurement errors. Although the simulation was not constrained to match the measured \( P_{O_2}S \) at the vessels, the simulated values are almost all very close to the measured values. The overall agreement between measured and simulated values supports the assumption that the \( P_{O_2} \) profiles are the outcome of the combined effects of diffusion and consumption in the regions under consideration.

The two-dimensional simulations give substantially lower estimates of consumption rate than the one-dimensional simulations (Table 1). In two-dimensional simulations, oxygen diffusing from the vessels has to supply tissue above and below the plane containing them (Fig. 2b), resulting in steeper \( P_{O_2} \) gradients around the vessels for a given consumption rate. Thus, a lower consumption rate gives best fit of the data compared to the one-dimensional simulations. Since the two-dimensional simulation is more exact, it probably leads to more reliable estimates than the one-dimensional solution.

The variation in the estimates of consumption is lower when two-dimensional simulations are used (Table 1). The coefficient of variation is reduced from 0.49 (one-dimensional) to 0.34 (two-dimensional). The highest estimates of \( M \) from the one-dimensional simulations occurred when the vessels were relatively close together (Table 1). In such cases, the amount of diffusion out of the plane of the vessels is relatively large; therefore, the estimates of \( M \) were most reduced in the two-dimensional simulations. As the distance between vessels increases, the error in assuming one-dimensional diffusion decreases.

The results of the two-dimensional simulations depend on the assumed thickness of the tissue. The actual thickness was not measured precisely but was known to be in the range 100 to 200 \( \mu \text{m} \). Therefore, we repeated several of the two-dimensional simulations assuming a tissue thickness of 200 \( \mu \text{m} \). In each case, the resulting estimates of consumption rate were very close to one-half of those shown in Table 1. This inverse relationship between assumed tissue thickness and estimated consumption rate is not surprising, since...
radial Po\(_2\) gradients near vessels depend on total oxygen consumption, which is proportional to the product of tissue thickness and consumption rate. The estimates assuming a tissue thickness of 200 \(\mu\)m represent lower bounds on the actual consumption, for two reasons: (a) the actual thickness of metabolizing tissue was less than 200 \(\mu\)m; and (b) microvessels deeper than 100 \(\mu\)m in the tissue could not be observed reliably in this tissue preparation. Such vessels may have contributed to oxygen delivery to deeper tissue. For these reasons, we base our estimates of oxygen consumption rate on an effective tissue thickness of 100 \(\mu\)m.

In the two-dimensional simulations, diffusion in the direction parallel to the vessels is assumed to be negligible. This approximation is valid if the segments bounding the tissue region are approximately parallel and straight, and the axial gradient in Po\(_2\) is approximately uniform. To assess the errors resulting from curved, non-parallel vessels, we carried out three-dimensional simulations for profile 11.1, which was obtained within a vascular loop, using the value for the consumption rate obtained from the two-dimensional simulation. The resulting Po\(_2\) profile is included in Fig. 3. The three-dimensional simulation results in a slightly shallower predicted profile than the two-dimensional simulation, with about a 10% reduction in the range of Po\(_2\) values. As might be expected, the vascular loop provides enhanced oxygen supply relative to two parallel straight vessels with a spacing equal to the width of the loop. However, the effect is slight, suggesting that the neglect of three-dimensional effects does not significantly affect the estimates of consumption rate.
Profiles 12.1 and 12.2 were obtained from adjacent regions of a single preparation, as were profiles 14.1 and 14.2. In each case, the two obtained estimates of local consumption differ by a factor of about two. The results, therefore, suggest that oxygen consumption shows significant local variations at the microregional level.

The estimated consumption rates are directly proportional to $D\alpha$. Some evidence suggests that $D\alpha$ is much higher in skeletal muscle than the values assumed here (11), but no similar evidence has been reported for tumor tissue.

In our analysis, $D\alpha$ was assumed to be the same in all regions. Actual values of $D\alpha$ may have varied from region to region, contributing to the variation in the estimated consumption rates. However, $D\alpha$ is unlikely to vary by a factor of two or more between regions, since the water content of tumor tissues (both intracellular and extracellular components) is relatively high, and oxygen diffusivity is primarily dependent on water content (2). Therefore, variation in $D\alpha$ is unlikely to account for most of the variation in estimated consumption rates.

In conclusion, the local rate of oxygen consumption is a key parameter in determining interstitial tumor tissue oxygenation. We have estimated local consumption rates in regions 87 μm to 286 μm wide lying between vessels, based on microelectrode measurements of $P_O_2$ profiles, and theoretical simulations of oxygen diffusion. The estimated consumption rates are in the range 0.8 to 2.2 cm$^{-2}$/100 g/min, consistent with previous estimates obtained from macroscopic tissue volumes (1). To our knowledge, these are the first microscale determinations of oxygen consumption in in vivo microvascular preparations. The method allows determination of consumption rate without the need to measure oxygen supply to and removal from the tissue by blood vessels. Furthermore, it permits investigation of microregional variations in consumption.

References


