Imaging of Oxygen Transfer Among Microvessels of Rat Cremaster Muscle

Hirosuke Kobayashi, MD, PhD; Naosada Takizawa, PhD

Background—The proximity of capillaries, arterioles, and venules provides complex spatial relationships that lead to oxygen transfer among microvessels. Although a conceptual image of complex oxygen transfer among microvessels has been hypothesized, in vivo mapping of oxygen saturation (SO₂) levels in microvessels had never been performed.

Methods and Results—The oxygen profile of the arterioles and venules of the rat cremaster muscle during normoxia and hypoxia was visualized by preparing pseudo-color images of SO₂ levels based on microspectrophotometry data obtained by using 3 different optical filters and a cooled CCD camera. The SO₂ images showed lower SO₂ levels in arterioles close to their walls, and the SO₂ levels in the paired venules showed higher SO₂ levels close to the arterioles. There were capillaries that crossed the microvessels whose SO₂ levels changed as they crossed the microvessels. The SO₂ levels were lower close to the vessel wall than in the centerline level of the microvessels, and the highest SO₂ levels in venules paralleling arterioles were skewed toward the arterial side. The SO₂ images showed that the SO₂ level in arterioles decreased after crossing venules, whereas the SO₂ level in venules increased after crossing arterioles.

Conclusions—Visualization of intravascular SO₂ levels suggested that oxygen is transferred between paired microvessels and moving between microvessels in rat cremaster muscle. The possibility that oxygen is transported from some arterioles to venules and tissue through adjacent capillaries is proposed. (Circulation. 2002;105:1713-1719.)

Key Words: imaging • microcirculation • oxygen • muscles

Previous studies have shown oxygen transfer from precapillary vessels to the tissues and into postcapillary vessels, as well as oxygen transfer among microvessels, and venules provide complex spatial relationships that lead to oxygen transfer among the microvessels. Although a conceptual image of complex oxygen transfer among microvessels has been described, in vivo oxygen saturation (SO₂) mapping of microvessels had never been performed.

In most previous in vivo studies, microphotometric measurements were made at the center of microvessels just before the bifurcation, with the assumption of thorough mixing of the blood before the bifurcation and homogeneous distribution of SO₂. Mott et al applied a newly developed optical triplicator for intravital video microscopy of oxygen saturation and reported findings of intravascular cross-sectional nonuniform oxygen saturation profiles, indicating the presence of luminal gradients in SO₂.

We obtained 3 microvascular images with 3 different optical filters and reconstructed images of SO₂ in cremaster microvessels based on the 3 images. Because the SO₂ images in microvessels were very complex, we focused on (1) spatial relationships in oxygen transfer between microvessels, particularly between paired arterioles and venules and between crossing (overridden and overriding) vessels, and (2) the intravascular cross-sectional SO₂ distribution in paired microvessels.

Methods

Animals

Animal care was in accordance with the guidelines of the Animal Care Committee of Kitasato University. Six male Wistar rats (Nihon SLC, Shizuoka, Japan) were intraperitoneally anesthetized with 1 g/kg urethane, intubated, and ventilated. An arterial catheter was introduced into a carotid artery to monitor mean arterial pressure (MAP). Data were collected only when 130 mm Hg > MAP > 90 mm Hg during normoxia and 120 mm Hg > MAP > 70 mm Hg at FiO₂ 0.12. Rectal temperature was monitored and maintained at 37.0±1.0°C with the use of a warm plate. The cremaster muscle was exposed and spread out according to Baez and then fixed on a transparent illumination stage (Microwarm plate DC-MP-300DM, Kitasato Supply) heated to 37°C. The muscle was covered with gas-impermeable film (Saran).

Apparatus and Calculations for SO₂ Imaging

Images were obtained by using a cooled charge-coupled device (CCD) camera (a 19×19-μm CCD chip with 512×512 pixels: TH7895B, Thomson CSP; cooling camera head: CH250, Photometrics) installed on a microscope (BH2, Olympus Optics) with a long-working-distance objective lens (SPAl x40,13, Olympus Optics). Samples were transilluminated through a long-distance focus condenser (BH2-CD, Olympus Optics) by a halogen lamp (12V/100W Olympus Optics) stabilized with an AC voltage stabilizer.
oxygenated or deoxygenated blood into the chamber, we then used 2 different types of chambers to validate our method. One was a 0.1-mm-thick gas-tight transparent chamber. After introducing the tonometer (IL237, Instrumentation Laboratory) for 30 minutes. We have performed experiments on rats. The other chamber was composed of 2 glass slides, with a 150-μm-thick coverglass placed between them at one end and the other ends touching. The open sides between the slides were sealed with hard wax. We then checked the thickness of the blood layer on the SO₂ measurements, and the thickness of the blood layer had no effect on the SO₂ values.

Validation of the Method

In Vitro Validation
Blood was obtained from male Wistar rats with a heparinized syringe and equilibrated with a gas mixture of 30% O₂ and 5% CO₂ with N₂ balance or a gas mixture of 5% CO₂ with N₂ balance by using a tonometer (IL237, Instrumentation Laboratory) for 30 minutes. We used 2 different types of chambers to validate out method. One was a 0.1-mm-thick gas-tight transparent chamber. After introducing the oxygenated or deoxygenated blood into the chamber, we then confirmed a 100% SO₂ homogenous image for fully oxygenated blood and a 0% SO₂ homogenous image for fully deoxygenated blood. The estimated absorbance (see Appendix) was identical to the observed absorbance.

The other chamber was composed of 2 glass slides, with a 150-μm-thick coverglass placed between them at one end and the other ends touching. The open sides between the slides were sealed with hard wax. We then checked the thickness of the blood layer on the SO₂ measurements, and the thickness of the blood layer had no effect on the SO₂ values.

In Vivo Validation
When resting (not stretched) cremaster muscle not covered with Saran was exposed to pure oxygen, the SO₂ in the microvessels was 100%. By euthanizing rats after ventilation with pure nitrogen and by superfusing the cremaster muscle with dithionite solution, SO₂ in the microvessels became 0%. To investigate changes in intravascular SO₂ after a change in tissue P O₂, we placed a particle of sodium hydrosulfite onto the muscle tissue 5 minutes after spot application of the sodium hydrosulfite. When resting (not stretched) cremaster muscle not covered with Saran was exposed to pure oxygen, the SO₂ in the microvessels was 100%. By euthanizing rats after ventilation with pure nitrogen and by superfusing the cremaster muscle with dithionite solution, SO₂ in the microvessels became 0%. To investigate changes in intravascular SO₂ after a change in tissue P O₂, we placed a particle of sodium hydrosulfite onto the muscle tissue 5 minutes after spot application of the sodium hydrosulfite.

Cross-Sectional SO₂ Profile in Paired Microvessels
Two adjacent pixels of an SO₂ image were averaged and these values were further averaged along the vascular axis for 200 μm in an attempt to reduce fluctuations in SO₂ values. The cross-sectional SO₂ profile thus was calculated. It should be noted that oxygen redistribution within the lumen in the radial direction might change the radial SO₂ profile along the longitudinal axis, but we evaluated the radial SO₂ profile averaged along this axis.

SO₂ Images of Microvascular Architecture
To obtain an overall view of the SO₂ levels in microvascular architecture, SO₂ images were pasted together one by one, and the SO₂ levels in microvascular architecture were reconstructed.

Visualization of Capillaries Crossing Paired Microvessels
One milliliter of 5g/dL FITC-albumin (Sigma) dissolved in normal saline was injected into the anesthetized animals via the arterial catheter, and capillaries crossing paired microvessels were photographed using a blue-range excitation filter coupled with an emission filter (BP495 and DM505, respectively, Olympus Optics).

Statistical Analysis
Values are expressed as means±SD. The Wilcoxon paired signed rank test was used to detect statistically significant differences between the SO₂ levels in microvessels and between the peak SO₂ position and centerline in microvessels.

Results

Intravascular SO₂ Profile of Paired Microvessels
The SO₂ images of paired arterioles and venules showed that the SO₂ levels on the venous side of the arterioles were lower than in the centerline and that the SO₂ levels on the arterial side of venules were higher than in the centerline (Figure 1).

Figure 1. Inhomogeneous intravascular SO₂ levels in paired microvessels. In paired arterioles and venules, SO₂ levels on the venous side of the arterioles (a) were lower than in the centerline, and SO₂ levels on the arterial side of venules (v) were higher than in the centerline. Bar=100 μm.

Figure 2. Capillaries crossing paired microvessels. Some capillaries were visible on the arterioles and venules as lines, and each line on the arterioles corresponded to a line on the venules (see arrowheads), indicating crossing capillaries. Bar=100 μm.
Some capillaries were visible on the arterioles and venules as lines, and each line on the arterioles corresponded to a line on the venules, indicating crossing capillaries (Figure 2).

The cross-sectional $SO_2$ distribution showed inhomogeneous intravascular $SO_2$ profiles, with intravascular $SO_2$ levels close to the vessel wall being lower than in the centerline (Figure 3). The $SO_2$ profiles of the venules were skewed toward the vessel wall on the arterial side (Figure 3).

The peak $SO_2$ level was higher ($P<0.05$) than the vessel wall $SO_2$ on either side of the arterioles, the same as in the venules, but the wall inner surface $SO_2$ levels on both sides did not differ. The peak arteriolar $SO_2$ level was higher than the peak venular $SO_2$ level ($P<0.05$). The position of the peak $SO_2$ in the venules was not in the centerline ($P<0.05$) but was significantly closer to the vessel wall on the arteriolar side ($P<0.05$) (Table).

Oxygen Transfer at Crossing Microvessels

The $SO_2$ levels in venules increased after crossing arterioles with higher $SO_2$ levels, and the $SO_2$ levels in arterioles decreased after crossing venules with lower $SO_2$ levels (Figure 4). The $SO_2$ levels in upstream venules were low and then increased after joining other venules with higher $SO_2$ levels. The $SO_2$ levels in upstream venules located close to an arteriole for a long distance were higher and decreased after joining other venules with lower $SO_2$ levels, but increased again after crossing an arteriole with a higher $SO_2$ level, whereas the $SO_2$ levels in upstream arterioles were high and decreased after crossing venules (Figure 5).

$SO_2$ Level in Microvascular Architecture

The $SO_2$ levels in microvessels changed along the vessels when they crossed other microvessels or were located close to
other microvessels, and the intravascular \( \text{SO}_2 \) levels were inhomogeneous (Figure 6).

**Visualization of Capillaries Crossing Paired Microvessels**

Capillaries crossed paired microvessels, particularly at the second- and third-order microvessels (Figure 7).

**Discussion**

The intravascular cross-sectional \( \text{SO}_2 \) profiles and \( \text{SO}_2 \) images of microvessels indicated that \( \text{SO}_2 \) declined toward the vessel wall. Previous studies\(^1\text{--}^4,16\) showed that during normoxia, \( \text{SO}_2 \) or \( \text{PO}_2 \) decreased downstream in arterioles, indicating a large oxygen efflux from the arterioles. The high rate of oxygen loss and uniformly lower values of blood \( \text{SO}_2 \) near the arterial wall indicate a substantial oxygen efflux from the arterioles. However, there has been controversy as to where the oxygen driven by this intravascular \( \text{PO}_2 \) gradient goes.

The oxygen disappearance rates measured in precapillary microvessels in vivo have been reported to be much higher than the theoretical estimates.\(^17\) One possible explanation for this is a high oxygen consumption rate of endothelial cells\(^16,18\); however, a theoretical study\(^19\) based on a large body of previous reports on endothelial metabolism and oxygen consumption failed to demonstrate a high oxygen disappearance rate from microvessels. Another possibility is overestimation of the oxygen efflux rate related to the assumption on which the estimate of convective oxygen flow was based, i.e., the assumption of cross-sectional uniformity of hemoglobin concentration and \( \text{SO}_2 \) within microvessels,\(^11\) especially because intravascular \( \text{SO}_2 \) is inhomogeneously distributed, with a high centerline level and low level near the vessel wall, as shown by Mott et al\(^12\) and in our present study. However, whether a more accurate estimate of the rate of oxygen disappearance results in lower values for oxygen efflux rates has never been evaluated.

Earlier observations\(^5,8\) showed that venous \( \text{SO}_2 \) can be higher than capillary \( \text{SO}_2 \). Our previous study\(^9\) showed that during normoxia, \( \text{SO}_2 \) in venous vessels paralleling arterioles was increased toward downstream (central) vessels. In our present study, the highest cross-sectional \( \text{SO}_2 \) levels in venules paralleling arterioles were skewed toward the arterial side, and the \( \text{SO}_2 \) images in the venules showed higher \( \text{SO}_2 \) levels close to the paired arterioles. These findings suggest arterio-venous oxygen transfer.

Although several lines of evidence have indicated that oxygen is transferred from arterioles to their paired venules, it is not obvious whether the oxygen transfer is solely diffusive. Some arterial and venous vessels are >100 \( \mu \text{m} \) apart, and diffusive oxygen transfer between these microvessels is unlikely. A mathematical model of paired arterioles and venules indicated that diffusive counter-current oxygen exchange would not normally be a significant effect.\(^20\)

There were several capillaries crossing some microvessels. The \( \text{SO}_2 \) images of the paired microvessels and the changes in \( \text{SO}_2 \) levels in the arterioles and venules with crossing capillaries suggest that these adjacent capillaries contributed to oxygen transfer between paired microvessels. It is possible that when capillaries cross an arteriole and then its paired venule, oxygen is transferred by diffusion from the arteriole to the capillaries, by convection in the capillaries, and subsequently by diffusion from the capillaries to the paired venule. These oxygenated crossing capillaries run closest to
the venules at the centerline of the venules (at the top or the bottom of the venules), and oxygen begins to be transferred from capillaries to venules before the centerline of the venules, resulting in a higher SO\(_2\) level just before the centerline.

The idea that oxygen loss by arterioles to adjacent capillaries might contribute to the high rate of oxygen loss by arterioles was explored theoretically in previous studies. Secomb and Hsu used a 3-dimensional configuration of capillaries and arterioles in a cuboid tissue region to simulate oxygen transfer from microvessels to tissue; they found that diffusive oxygen loss from arterioles equaled \(\frac{1}{10}\) of consumption in the tissue, and that crossing capillaries absorbed much of this oxygen (\(\approx 45\%\) of consumption) and delivered it at downstream locations. Thus, diffusive oxygen transfer from arterioles to capillaries seemed to play an important role in distributing oxygen throughout the tissue.

Accordingly, it is theoretically and experimentally likely that a considerable part of the oxygen that left some arterial microvessels was transferred to crossing capillaries and was carried by the capillaries to venules and tissue.

Oxygen transfer from arterioles to crossing venules was also shown in this study, and thus it is likely that crossing microvessels reduce the barrier to diffusional oxygen transfer, making intervessel oxygen transfer easier. Oxygen would transfer from normoxic arterioles to hypoxic venules, and the venules would in turn become normoxic and would provide oxygen to the tissues.

In conclusion, the intravascular SO\(_2\) images suggested oxygen transfer among microvessels in muscle. We propose...
the possibility that a considerable part of the oxygen that disappears from some arterioles is transferred to venules and tissue through adjacent capillaries.

Appendix

An image file was obtained using each of 3 optical filters i (1, 2, or 3). The peak transmission wavelengths (λ) of the filters were: λ1 = 521 nm, λ2 = 546 nm, and λ3 = 555 nm. The transmission profile of each optical filter, F(i, λ), was measured. F(i, λ) was defined as the ratio of the transmitted photon number to inlet photon number at the wavelength of λ. Each image file obtained using the optical filter, i, was expressed as E(i)=m(i) s(i), where m(i) is the number of photons captured at a CCD element of j in 1 second. The subscript j indicates the location on the CCD chip, and the CCD elements consisted of 512 columns and 512 rows, the element j therefore ranging from 1 to 262 144. The photon number of each element in 1 second was stored as a 16-bit digit.

In order to obtain an S02 image, S[i,j], where i is the S02 level at each element j, we captured 3 reference (blank) images, R[i,j]=m(i) s(i), using each optical filter i without specimen. The observation field was homogeneously illuminated by the Koller illumination system with an AC voltage stabilizer. As a result, the density of each reference image was flat, and R[i,j]=m(i) s(i) was expressed as R[i,j]=m(i) s(i). We then obtained 3 images, E(i)=m(i) s(i), using each optical filter, of rat cremaster muscle with microvessels.

Each element of the absorbance image, A[i,j], using an optical filter, i, was then calculated as follows:

\begin{equation}
A_{i,j} = \log_{10}(F/m_{i,j})
\end{equation}

The extinction coefficients of both oxygenated and deoxygenated blood using an optical filter, i, are influenced by the transmission profile of the filter.

When the reference light intensity and the transmitted light intensity through fully oxygenated unit blood (1 unit corresponds to 1 mol/L hemoglobin with 1 cm blood layer) at wavelength λ without a filter are set at r(λ) and q(λ), respectively, the reference light intensity, r(λ), and the transmitted light intensity through the fully oxygenated unit blood layer, q(λ), are expressed as:

\begin{equation}
r(\lambda) = \int_{\lambda-\Delta}^{\lambda+\Delta} r(\lambda) F(\lambda) d\lambda
\end{equation}

and

\begin{equation}
q(\lambda) = \int_{\lambda-\Delta}^{\lambda+\Delta} q(\lambda) F(\lambda) d\lambda
\end{equation}

Similarly, if the transmitted light intensity through fully deoxygenated unit blood layer, q(λ), then

\begin{equation}
q_{deoxy} = \int_{\lambda-\Delta}^{\lambda+\Delta} q_{deoxy}(\lambda) F(\lambda) d\lambda
\end{equation}

When ε(λ) and ε(λ) are molar extinction coefficients of the fully oxygenated and deoxygenated blood unit, respectively, at a wavelength λ, we obtain

\begin{equation}
\epsilon_{oxy}(\lambda) = \log_{10}(r(\lambda)/q_{oxy}(\lambda))
\end{equation}

and

\begin{equation}
\epsilon_{deoxy}(\lambda) = \log_{10}(r(\lambda)/q_{deoxy}(\lambda))
\end{equation}

Combining Equations 3 and 5, and combining Equations 4 and 6, we obtain

\begin{equation}
q_{deoxy} = \int_{\lambda-\Delta}^{\lambda+\Delta} (r(\lambda) F(\lambda)/10^{\epsilon_{deoxy}(\lambda)}) d\lambda
\end{equation}

Using an optical filter, i, with a peak transmission wavelength of λ, the molar extinction coefficients of fully oxygenated and deoxygenated blood layer, E(oxy) and E(deoxy), accounting for the filter characteristic F(λ), can be obtained as,

\begin{equation}
E_{oxy} = \log_{10}(r(\lambda)/q_{oxy})
\end{equation}

and

\begin{equation}
E_{deoxy} = \log_{10}(r(\lambda)/q_{deoxy})
\end{equation}

In our previous study we obtained ε(oxy)(λ) and ε(deoxy)(λ) and found that ε(oxy)(λ) and ε(deoxy)(λ) were identical to previously reported values at several discrete wavelengths. Using these ε(oxy)(λ), ε(deoxy)(λ) and r(λ) values, E(oxy) can be obtained by Equations 2, 7, and 9, and E(deoxy) by Equations 8, 10.

The integration was performed by numerical integration with a wavelength step of 0.56 nm, which was the resolution limit of the spectrophotometry in our previous study, ranging ±28 nm, because F(λ) was zero beyond this range. E(oxy) and E(deoxy) thus obtained were:

\begin{equation}
E_{oxy} = 0.59 and E_{deoxy} = 0.50 at \lambda = 521 nm
\end{equation}

\begin{equation}
E_{oxy} = 0.95 and E_{deoxy} = 1.06 at \lambda = 546 nm
\end{equation}

\begin{equation}
E_{oxy} = 0.82 and E_{deoxy} = 1.09 at \lambda = 555 nm
\end{equation}

The absorbance (a) at position j of a microvessel at wavelength λ is formulated as follows:

\begin{equation}
a_{ij} = \log_{10}(r(\lambda)/m_{ij})
\end{equation}

where c is the value of the hemoglobin concentration multiplied by the mean optical path length at the measured pixel of the microvessel, b is a scattering term, and s_i is the S02 level (%). Applying a filter i and accounting for the transmission profile of the filter, the absorbance at the position j obtained using filter i, a_{ij}, can now be formulated as,

\begin{equation}
a_{ij} = \log_{10}(r(\lambda)/m_{ij})
\end{equation}

These 3 unknown parameters (s, c, and b) at a position j could be obtained by solving the 3 equations for i = 1, 2, and 3. Therefore,
where

\[ a_{i,j}^{1,2,j} = \frac{\Delta S_{o_2}^{i,j} - \Delta S_{o_2}^{k,j}}{100} \]

and

\[ E_{\text{oxy}}^{i,j} = E_{\text{oxy}}^{i} - E_{\text{oxy}}^{j} \]

and

\[ E_{\text{deoxygen}}^{i,j} = E_{\text{deoxygen}}^{i} - E_{\text{deoxygen}}^{j} \]

for \( i, k = 1, 2, \text{ or } 3 \).

This yielded the \( S_{o_2} \) image, \( S_{\{s_{i,j}\}} \).

References


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