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A computational model for nitric oxide, nitrite and nitrate biotransport in the microcirculation: effect of reduced nitric oxide consumption by red blood cells and blood velocity

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Abstract

Bioavailability of vasoactive endothelium-derived nitric oxide (NO) in vasculature is a critical factor in regulation of many physiological processes. Consumption of NO by RBC plays a crucial role in maintaining NO bioavailability. Recently, Deonikar et al (Deonikar and Kavdia, 2009b) reported a effective NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ that is ~7 times lower than the commonly used NO-RBC reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. To study the effect of lower NO-RBC reaction rate constant and nitrite and nitrate formation (products of NO metabolism in blood), we developed a 2D mathematical model of NO biotransport in 50 and 200 μm ID arterioles to calculate NO concentration in radial and axial direction in the vascular lumen and vascular wall of the arterioles. We also simulated the effect of blood velocity on NO distribution in the arterioles to determine whether NO can be transported to downstream locations in the arteriolar lumen. The results indicate that lowering the NO-RBC reaction rate constant increased the NO concentration in the vascular lumen as well as the vascular wall. Increasing the velocity also led to increase in NO concentration. We predict increased NO concentration gradient along the axial direction with an increase in the velocity. The predicted NO concentration were 281–1163 nM in the smooth muscle cell layer for 50 μm arteriole over the blood velocity range of 0.5–4 cm/s for $k_{\text{NO-RBC}}$ of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, which are much higher than the reported values from earlier mathematical modeling studies. The NO concentrations are similar to the experimentally measured vascular wall NO concentration range of 300–1000 nM in several different vascular beds. The results are significant from the perspective that the downstream transport of NO is possible under right circumstances.

Keywords

Nitric oxide; biotransport; mathematical model; red blood cell; diffusion; convection; microcirculation

Introduction

Nitric oxide (NO), a potent vasodilator, is generated via catalytic conversion of L-arginine by endothelial nitric oxide synthase (eNOS) in vascular beds (Furchgott and Zawadzki, 1980; Ignarro et al., 1987). Vascular bioavailability of NO is a critical factor in regulation of many

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physiological processes including blood pressure (Moncada et al., 1991), vascular tone (Kelm et al., 1995), vascular permeability (Fukumura et al., 1997), adhesion and aggregation of platelets (Radomski et al., 1991; Radomski et al., 1993), and smooth muscle cell proliferation (Marks et al., 1995). A lack of NO bioavailability leads to several pathophysiological conditions such as atherosclerosis, renal failure, high blood pressure, and diabetes-related vascular complications.

NO homeostasis in the vasculature is mainly regulated by the shear stress-dependent NO production from endothelial cells and NO consumption red blood cells (RBC). In the smooth muscle cells (SMC), NO activates sGC that converts GTP to cyclic-GMP leading to SMC relaxation (Arnold et al., 1977; Furchgott and Zawadzki, 1980; Ignarro et al., 1987; Palmer et al., 1987). Majority of endothelium-derived NO enters the vascular lumen (Butler et al.) where NO reacts rapidly with RBC-Hb and forms nitrate (NO_3^-), methemoglobin (metHb), and S-nitrosohemoglobin (SNOHb) with oxyhemoglobin (oxyHb) or forms nitrosyl hemoglobin (HbNO) with deoxyhemoglobin (deoxyHb) (Cassoly and Gibson, 1975; Eich et al., 1996). In addition, a small amount of NO is converted to nitrite (NO_2^-) from autooxidation (Ignarro et al., 1993).

Experimental studies have reported an in vivo NO concentration in the range of 300–1000 nM in vascular wall (Bohlen et al.; Chen et al., 2008b; Tsai et al., 2005; Tsai et al., 2006; Vukosavljevic et al., 2006; Zani and Bohlen, 2005). This poses a paradox that how NO is able to escape scavenging by large amount of RBC-Hb present in close proximity of its site of production (i.e. endothelium) and still be able to maintain 100's of nanomolar levels in the vessel wall. One way of resolving this paradox is to evaluate the NO consumption in the vascular lumen. For this purpose, NO-RBC interaction has been the focus of several recent novel experimental approaches and theoretical and computational modeling studies (Azarov et al., 2005; Deonikar and Kavdia, 2009a; Deonikar and Kavdia, 2009b; Han and Liao, 2005; Joshi et al., 2002; Liao et al., 1999; Liu et al., 1998; Liu et al., 2002; Vaughn et al., 2001). These studies reported that the NO consumption by RBCs ($\sim 10^5 \text{ M}^{-1}\text{s}^{-1}$) is two orders of magnitude lower compared to free Hb ($\sim 10^7 \text{ M}^{-1}\text{s}^{-1}$).

The reduction in NO consumption by RBC as compared to free Hb is attributed to several transport/diffusional resistances for NO transport in the vascular lumen. These resistances include 1) a diffusional resistance from the cell free layer near vessel wall (Liao et al., 1999), 2) a diffusional resistance from the unstirred layer surrounding a single RBC (Liu et al., 1998; Liu et al., 2002), and 3) an intrinsic resistance offered by the submembrane skeleton of RBCs (Deonikar and Kavdia, 2009a; Han and Liao, 2005; Vaughn et al., 2000). We reported a NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ for 5–40% hematocrit (Deonikar and Kavdia, 2009b). The NO-RBC reaction rate constant is ~ 7 -fold lower than the widely used reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ (Carlsen and Comroe, 1958), which was measured at less than 1% hematocrit. This further reduction in NO-RBC reaction rate is attributed to both external and membrane diffusional resistances (Deonikar and Kavdia, 2009a; Deonikar and Kavdia, 2009b).

In addition to a reduction in NO-RBC reaction rate, NO bioactivity is preserved in the form of SNOHb (Jia et al., 1996) or nitrite (Crawford et al., 2006) after entering the vascular lumen. SNOHb transfers NO in the form of low molecular weight thiols which exports NO to cause vasodilation (Diesen et al., 2008; Jia et al., 1996; Stamler et al., 1997). DeoxyHb can act as nitrite reductase to generate NO from nitrite which is exported as NO or unreactive NO species including nitrous anhydride to cause vasodilation (Basu et al., 2007; Crawford et al., 2006; Gladwin et al., 2006; Vitturi et al., 2009).

The mechanisms of NO transport from vascular lumen to the SMC are under intense investigations. Mathematical models of NO biotransport in the microcirculation have provided detailed NO profiles in wide range of important physiologic and hemodynamic parameters (Chen et al., 2007b; Chen et al., 2006; Huang et al., 2007; Kavdia and Popel, 2003; Kavdia and Popel, 2006; Kavdia et al., 2002; Lancaster, 1994; Tsoukias, 2008; Vaughn et al., 2001; Vaughn et al., 1998b). All of these models have used a NO-RBC reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. Kavdia and Popel (Kavdia and Popel, 2003) reported NO levels of $\sim 100 \text{ nM}$ in the smooth muscle layer. Additionally, the venule in a paired arteriole-venule (Kavdia and Popel, 2006) and nNOS (Kavdia and Popel, 2004) can also be important contributor to the NO concentration in arteriolar wall. Because of a high Damkohler number, which represents the ratio of diffusion to convection, convective transport of NO was assumed negligible (Chen et al., 2007b; Chen et al., 2006; Kavdia and Popel, 2003; Kavdia and Popel, 2006; Tsoukias, 2008; Vaughn et al., 1998a). However, a mathematical model describing NO transport in the axial direction through parallel flat plates (Smith et al., 2003) and a recent experimental study (Bohlen et al., 2009) reported that NO could be transported from upstream to downstream locations indicating importance of convective transport of NO in the vascular lumen.

Only recently, mathematical models started the examination of the extent of contribution of NO generated by transnitrosation of SNOHb or nitrite reduction by deoxy hemoglobin in the vascular lumen (Chen et al., 2008a; Chen et al., 2007a; Liu et al., 2007). Chen et al. (Chen et al., 2008a; Chen et al., 2007a) reported that SNOHb or nitrite alone will generate 0.25–260 pM NO concentrations at the smooth muscle layer. Liu et al. (Liu et al., 2007) reported that the NO production rate of $80 \mu\text{M/s}$ is needed to achieve steady state NO concentrations of 10 nM 's in the blood stream; however, this high NO production rate would rapidly oxidize all blood Hb. Jourdeuil et al. (Jourdeuil et al., 2000) and Bohlen et al. (Bohlen et al., 2009) reported that the nitrosothiols and free NO, respectively can be transported downstream in the microcirculation.

In order to understand the effect of reduction of NO RBC reaction rate to $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (Deonikar and Kavdia, 2009b) and downstream transport of free NO (Bohlen et al., 2009), we developed a two dimensional computational model for NO biotransport in arterioles of size 50 and $200 \mu\text{m}$ in this study. We estimate the effect of NO-RBC reaction rate and blood velocity on NO transport both in vessel wall and downstream directions. In addition, we estimate the formation of nitrite-a product of NO autooxidation, and nitrate-a product of NO-RBC interaction, in the vascular lumen.

Computational modeling of NO biotransport in microcirculation

A computational model representing NO biotransport in arterioles of $50 \mu\text{m}$ and $200 \mu\text{m}$ diameters was developed to study the radial and axial NO distribution in the microcirculation.

Model geometry

Each arteriole is represented in cylindrical geometry. The model geometry consists of concentric cylinders representing different regions of an arteriole (Fig. 1) which include: cell rich lumen, cell free layer near the vessel wall, endothelium, interstitial space, smooth muscle cell layer, and non-perfused and perfused parenchymal tissue. The cell rich layer in the arteriolar lumen is assumed to consist of uniformly distributed RBCs. An effective reaction rate constant for NO-RBC reaction, which is a function of extracellular diffusional resistance and RBC membrane permeability, accounts for NO consumption by RBCs. NO production from the endothelium is considered as a linear function of shear stress on the vessel wall arising from the blood flow in the vessel lumen (Kavdia and Popel, 2003).

Governing equations

A steady state mass balance equation based on the diffusion, convection and reaction kinetics of NO in each region of the arteriole is written below.

$$\frac{1}{r} D_{NO} \frac{\partial}{\partial r} \left(r \frac{\partial C_{NO}}{\partial r} \right) - \sum R_{NO} + \sum q_{NO} = v_z \frac{\partial C_{NO}}{\partial z} \quad (1)$$

where D_{NO} represents diffusivity of NO, C_{NO} represents NO concentration, R_{NO} is the sum of NO reaction rates, q_{NO} is the sum of NO production rates. Average velocity of blood (v_z) is equal to $v_z = \left(1 - \frac{r^2}{R^2}\right) \times v_{zmax}$, where v_{zmax} is the maximum velocity of blood (at $r = 0$), and R is the inner radius of the vessel.

Boundary conditions

Boundary conditions of zero flux are applied at the center of the vessel and at the outer surface of perfused tissue. Boundary conditions of continuity of NO flux and NO concentration were applied at all other interfaces except endothelial interfaces. To reflect the NO production at the endothelial layer from the membrane bound eNOS, the following boundary conditions representing surface release of NO are applied at the endothelium-cell free zone and endothelium-interstitial space interfaces. At endothelium-cell free zone interface:

$$\left. \frac{\partial C_{NO}}{\partial r} \right|_{\text{cell_free_zone}} - \left. \frac{\partial C_{NO}}{\partial r} \right|_{\text{endothelium}} = P_{NO_endo} / D_{NO} \quad (2)$$

At endothelium-interstitial space interface:

$$\left. \frac{\partial C_{NO}}{\partial r} \right|_{\text{endothelium}} - \left. \frac{\partial C_{NO}}{\partial r} \right|_{\text{interstitium}} = P_{NO_endo} / D_{NO} \quad (3)$$

where P_{NO_endo} is the NO production from the endothelium.

Sources of NO production

NO release from endothelium is reported to be proportional to the shear stress (Kanai et al., 1995; Kavdia and Popel, 2003). The proportionality of NO production and shear stress is given as follows.

$$P_{NO_endo} = P_{NO_control} \times \frac{\tau}{\tau_{control}} \quad (4)$$

where $P_{NO_control}$ is 5.3×10^{-12} moles $\text{cm}^{-2} \text{s}^{-1}$ and $\tau_{control}$ is 24 dyne cm^{-2} at physiological blood velocity of 0.5 cm/s and blood viscosity of 3 cP (Kavdia and Popel, 2003; Vaughn et al., 1998b). As mentioned above, the shear stress dependent NO production is applied as the boundary condition at the endothelium-cell free zone and endothelium-interstitial interfaces.

NO production by the capillary endothelium is assumed the same as that of the NO production from arteriolar endothelium. NO release from capillary endothelium (q_{NO_cap}) is calculated

from $q_{NO_cap} = \text{total inner surface area of capillaries} \times \frac{P_{NO_endo}}{\text{Volume of arteriole}}$. The total inner surface

area of capillaries is obtained from capillary density of $14.35/\text{cm}^2$ and capillary radius of $1.8 \mu\text{m}$ (Ellsworth et al., 1988). Capillary NO production is estimated to be $8.6 \times 10^{-7} \text{ M/s}$. There is no reported direct relationship between capillary endothelial NO production and shear stress. We assumed the capillary NO production to be independent of arteriolar wall shear stress.

NO consumption reactions

NO reacts with RBC-Hb and oxygen in the vascular lumen. The reaction rate is

$$R_{\text{NO}} = k_{\text{NO-RBC}} C_{\text{RBC-Hb}} C_{\text{NO}} + k_{\text{NO-O}_2} C_{\text{O}_2} C_{\text{NO}}^2 \quad (5)$$

where $k_{\text{NO-RBC}}$ is the effective reaction rate constant for NO-RBC reaction, $C_{\text{RBC-Hb}}$ is the concentration of RBC-Hb per heme basis. $k_{\text{NO-O}_2}$ is the reaction rate constant for NO autooxidation reaction (second order reaction in C_{NO}) (Lewis and Deen, 1994) and C_{O_2} is the concentration of oxygen. In the cell free zone, we assumed zero hematocrit.

In the endothelial, interstitial and the non-perfused parenchymal tissue regions, the rate of NO consumption is

$$R_{\text{NO}} = k_{\text{NO-O}_2} C_{\text{O}_2} C_{\text{NO}}^2 \quad (6)$$

In the smooth muscle cells, the rate of NO consumption by sGC is

$$R_{\text{NO}} = k_{\text{sGC-NO}} C_{\text{NO}}^2 \quad (7)$$

where $k_{\text{sGC-NO}}$ is the second order reaction rate constant for sGC-NO reaction (Vaughn et al., 1998b).

In the capillary-perfused tissue region, NO will react with the capillary RBC-Hb and oxygen and the rate of NO consumption is

$$R_{\text{NO}} = k_{\text{cap}} C_{\text{NO}} + k_{\text{NO-O}_2} C_{\text{O}_2} C_{\text{NO}}^2 \quad (8)$$

where k_{cap} is the effective reaction rate constant for NO-RBC reaction in the capillaries and is based on the capillary hematocrit and fractional capillary volume.

Nitrite and Nitrate Concentration Profiles in the Vascular Lumen

Nitrite is formed as a result of NO autooxidation reaction. Reaction of NO with oxyHb results in the formation of nitrate and methemoglobin. The steady state mass balance equations of nitrite and nitrate are given below.

Nitrite concentration profile:

$$v_z \frac{dC_{\text{NO}_2^-}}{dz} = k_{\text{NO-O}_2} C_{\text{NO}}^2 C_{\text{O}_2} \quad (9)$$

Nitrate concentration profile:

$$v_z \frac{dC_{\text{NO}_3^-}}{dz} = k C_{\text{NO}} C_{\text{RBC-Hb}} \quad (10)$$

Nitrite and nitrate concentrations were assumed zero at $z=0$ for all r .

Mixing cup concentrations of NO, nitrite and nitrate

The mixing cup concentrations of NO, nitrite and nitrate exiting the vessels were obtained from the respective NO, nitrite and nitrate concentration profiles at $z=L$ (length of the vessel) using the following equation.

$$C_{i,\text{mixing cup}} = \frac{\int_0^R \left\{ \left(1 - \frac{r^2}{R^2} \right) C_i^{z=L} \right\} dr}{\int_0^R \left\{ \left(1 - \frac{r^2}{R^2} \right) \right\} dr} \quad (11)$$

where $i = \text{NO}, \text{NO}_2^- \text{ or } \text{NO}_3^-$

Model parameters

The parameter values are listed in Table 1. Simulations were performed for arterioles of 50 and 200 μm inner diameters. The lengths of the arteriolar segments were 500 and 2000 μm for 50 and 200 μm arteriole, respectively. This resulted in the same diameter to length ratio of 1:10 for both the arterioles. The blood velocities used in the simulations were 0.1, 0.5, 1 and 4 cm/s for both the arterioles.

Based on the hematocrit and vessel diameter, the thickness of cell free layer was 4.5 and 4 μm for 50 and 200 μm arteriole, respectively (Sharan and Popel, 2001). Endothelium and interstitial regions were assumed to be 0.5 μm thick (Kavdia et al., 2002; Walmsley et al., 1982) for both the arterioles. The smooth muscle thickness was 6 μm for 50 μm ID arteriole (Haas and Duling, 1997) and 15 μm for 200 μm ID arteriole (Bevan et al., 1999). The non-perfused tissue region thickness was 30 μm . For both the arterioles, the total distance from the center to the outer parenchymal surface was 500 μm ; the simulations showed that the NO concentration reached a steady state value in less than 200 μm distance from the vessel center.

For all the regions in both arterioles, the NO diffusivity was $3.3 \times 10^5 \text{ cm}^2/\text{s}$ (Malinski et al., 1993). NO autooxidation reaction rate constant was $9.6 \times 10^6 \text{ M}^{-2} \text{ s}^{-1}$ (Lewis and Deen, 1994). Based on the in vivo oxygen partial pressure of 15.5 mmHg, value of CO_2 was $27 \times 10^{-6} \text{ M}$ (Kavdia and Popel, 2003; Popel, 1989). The reaction rate constant for NO-sGC reaction was $5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ (Vaughn et al., 1998b). Systemic hematocrit of 45% was used in the RBC core region. At 20.3 mM concentration of Hb (heme basis) in each RBC, the total RBC-Hb concentration was calculated to be 9.135 mM in the RBC core region for 45% hematocrit. The value of capillary hematocrit was assumed 30% (Kavdia et al., 2002).

We recently reported much lower effective NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (Deonikar and Kavdia, 2009b) as compared to the previously used effective reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (Carlsen and Comroe, 1958). To study the effect of lower NO-RBC reaction rate constant on NO bioavailability in microcirculation, we performed the simulations for effective NO-RBC reaction rate constants of 0.2×10^5 , 0.5×10^5 and $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. Based on the hematocrit, fractional capillary volume and NO-RBC effective reaction

constants, the capillary NO-RBC reaction rate constants (k_{cap}) were estimated to be 1.77, 7.11 and 12.4 s^{-1} .

Numerical Methods

Steady state nitric oxide mass balance equation (Eq. 1) was solved numerically with appropriate boundary conditions using FlexPDE5 software (PDESolutions Inc., Antioch, CA). The software's adaptive meshing algorithm feature generates finite elements in proportion with the concentration gradients in the different regions (higher the concentration gradient, more the number of elements) as shown in Fig. 1b. Relative accuracy used for all the simulations conducted in this study was 0.005.

Results

Effect of blood flow and lower NO-RBC reaction rate constant on steady state NO concentration profiles in arterioles

We recently reported effective NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (Deonikar and Kavdia, 2009b) which is lower than the commonly used effective NO-RBC reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ reported for 1% hematocrit (Carlsen and Comroe, 1958). To analyze the effect of the lowered effective NO-RBC reaction rate constant, we predicted the steady state NO concentration profiles for the tissue segment containing arterioles of 50 and 200 μm diameter for three effective NO-RBC reaction rate constants of 0.2×10^5 , 0.5×10^5 and $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. Endothelial NO production rate of $5.3 \times 10^{-12} \text{ mol cm}^{-2} \text{ s}^{-1}$ (Vaughn et al., 1998b) was used for both arterioles. The NO production was assumed directly proportional to the wall shear stress. The simulations were performed for blood velocity values of 0.1, 0.5, 1 and 4 cm/s. Figure 2 shows the steady state NO concentration profiles in 50 μm diameter arteriole (panels A, B and C) at the 0.05 cm length and in 200 μm diameter arteriole (panels D, E and F) at the 0.2 cm length.

For both the arterioles, a lower NO-RBC reaction rate constant led to an increase in NO concentrations in the luminal as well as abluminal regions. For all reaction rate constant, the NO concentrations were higher for 50 μm vessel as compared to that of for 200 μm vessel. The steady state parenchymal tissue NO concentrations increased ~7–8 times for both arterioles for all velocity when the reaction rate constant was decreased from $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ to $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. For 50 μm arteriole at the center in the vessel lumen, NO concentrations were 2.9, 5.6, 9.0 and 45 nM at velocity of 0.1, 0.5, 1, and 4 cm/s, respectively for the NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. The NO concentrations were less than 0.08 nM for all velocities at the NO-RBC reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$.

Distribution of NO, nitrite and nitrate in the lumen

Figure 3A and B show the NO concentration profiles in the vascular lumen of arterioles of 50 μm dia and 500 μm length and 200 μm dia and 2000 μm length, respectively. In Figure 3, panels A–D, E–H and I–L represent simulation results from the NO-RBC reaction rate constant of 1.4×10^5 , 0.5×10^5 and $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively. For the same blood velocity, NO concentrations were higher for 50 μm ID arteriole as compared with the NO concentrations for 200 μm ID arteriole.

The endothelial NO transports through the cell free layer and reaches cell rich regions of vascular lumen of both the arterioles. There is an axial gradient of NO concentration along the vessel length in the direction of blood flow (Figure 3). As the blood velocity was increased, the axial NO concentration gradient in the vascular lumen increased for all of the NO-RBC reaction rate constants. The highest axial gradient is for blood velocity of 4 cm/s and NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (seen in Panel L of Figure 3A and 3B). The axial

accumulation of NO indicates downstream transport of NO in the vessel lumen resulting from convective transport of NO in the vascular lumen.

Nitrite concentration profiles in the vascular lumen of both the arterioles were calculated using Eq. 9. Figure 4A and 4B show the nitrite concentration profiles for 50 and 200 μm diameter arterioles, respectively. Panels A–D, E–H and I–L in Figure 4 represent nitrite levels in the vascular lumen for NO-RBC reaction rate constant of 1.4×10^5 , 0.5×10^5 and $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively. Nitrite concentrations for both the arterioles increased with decrease in NO-RBC reaction rate constant and increase in blood velocity. The results predict a nitrite concentration gradient in the direction of blood flow. The nitrite concentration increased with a decrease in the NO-RBC reaction rate constant.

Figure 5A and 5B show the nitrate concentration profiles obtained for 50 and 200 μm diameter arterioles, respectively. Panels A–D, E–H and I–L in Figure 5 show the nitrate levels for NO-RBC reaction rate constant of 1.4×10^5 , 0.5×10^5 and $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively. Nitrate concentration decreased with a decrease in NO-RBC reaction rate constant and an increase in blood velocity. However, we observed that for the low blood velocity of 0.1 cm/s, the nitrate concentrations was the highest for NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. For NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, the NO concentration in the vascular lumen is high due to a low NO consumption (see Figure 3 panel A and I). In addition, NO diffuses to longer distances compared to higher NO-RBC reaction rates. Nitrate concentrations are a function of local NO concentrations, NO-RBC reaction rates and the Hb concentration. Therefore, although the NO-RBC reaction rate is low, the nitrate concentrations are higher resulting from its multiplication with several fold higher NO concentrations at the 0.1 cm/s velocity.

Flow mediated modulation of endothelial and smooth muscle cell NO concentrations

Steady state NO concentrations at the endothelium and at the smooth muscle cell are plotted as a function of blood velocity in Figure 6. The NO concentrations at the endothelium and smooth muscle layer increased linearly with blood velocity for 50 μm diameter arteriole (panels A & B) and 200 μm ID arteriole (panels C & D) for all NO-RBC reaction rate constants. A lower NO-RBC reaction rate has a higher increase in the NO concentration for an increase in velocity as shown by the higher slope of the lines in Figure 6. For 50 μm diameter arteriole, as the blood velocity increased from 0.1 cm/s to 4 cm/s the endothelium NO concentrations increased in the range of 32.3–750.9 nM, 62.1–924.1 nM, and 125.5–1229.8 nM for $k_{\text{NO-RBC}}$ of 1.4×10^5 , 0.5×10^5 and $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively. Over the same range of blood velocity, the smooth muscle NO concentrations increased in the range of 41.2–641.4 nM, 87.6–826.9 nM, and 180.6–1162.8 nM for $k_{\text{NO-RBC}}$ of 1.4×10^5 , 0.5×10^5 and $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively.

For 200 μm diameter arteriole, as the blood velocity increased from 0.1 to 4 cm/s the endothelium NO concentrations increased in the range of 25.9–722.8 nM, 36.2–851.1 nM, and 86.8–1087.4 nM for $k_{\text{NO-RBC}}$ of 1.4×10^5 , 0.5×10^5 and $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively. The smooth muscle NO concentrations increased in the range of 37.5–531.8 nM, 59.4–650.7 nM, and 174.9–953.1 nM for $k_{\text{NO-RBC}}$ of 1.4×10^5 , 0.5×10^5 and $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively, as the velocity increased from 0.1 to 4 cm/s.

Effect of NO-RBC reaction rate constant and blood velocity on exiting (mixing cup) NO, nitrite and nitrate concentrations in the arterioles

The mixing cup concentration of NO, nitrite and nitrate exiting the arteriolar segment were calculated from the NO, nitrite and nitrate concentration profiles in the vascular lumen using Eq 11. The mixing cup concentrations are plotted as a function of blood velocity and

$k_{\text{NO-RBC}}$ in Figure 7. The NO, nitrite and nitrate concentrations are shown in panel A–C, respectively for 50 μm arteriole and panel D–F, respectively for 200 μm arteriole.

For all $k_{\text{NO-RBC}}$ and blood velocities, the mixing cup NO concentration exiting 50 μm arteriole was higher than that of for 200 μm arteriole. The mixing cup NO concentrations increased linearly with blood velocity for all $k_{\text{NO-RBC}}$. The mixing cup NO concentrations exiting arteriolar segments are in the range of 0.17–236 nM. For a given blood velocity, the mixing cup NO concentrations increased with decrease in $k_{\text{NO-RBC}}$ because of a reduced NO uptake by RBCs.

The mixing cup nitrite concentration exiting the arteriolar segments varied from 0.03 to 2.41 μM . The mixing cup nitrite concentration increased with a decrease in $k_{\text{NO-RBC}}$ for both the arterioles at all velocities. A possible explanation for the increase in nitrite formation is higher available NO for its reaction with oxygen. For all $k_{\text{NO-RBC}}$, the mixing cup nitrite concentration increased with blood velocity for all velocities except for $k_{\text{NO-RBC}}$ of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ at velocity of 0.1 cm/s. At the low blood velocity of 0.1 cm/s, NO concentrations in the vascular lumen are high for NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. This results in higher nitrite formation from NO auto-oxidation. However, as the blood velocity increases to 0.5 cm/s, for the same reaction rate constant, nitrite production drops because of low residence time for NO auto-oxidation reaction. As the blood velocity increases from 0.5 cm/s, even though the residence time decreases for NO auto-oxidation reaction, the NO availability increases because of increased NO production at higher velocity (as seen in Fig. 3A & B), resulting in higher nitrite formation.

The mixing cup nitrate concentration exiting the arteriolar segments varied from 1.03 to 5.9 μM . The mixing cup nitrate concentration decreased with increase in blood velocity for all the $k_{\text{NO-RBC}}$ in both the arterioles because of the reduction in residence time for NO-RBC reaction with increase in blood velocity.

Discussion

In this study, we developed a cylindrical geometry mathematical model that incorporates radial and axial transport of NO in arterioles to explore the possibility of downstream transport of NO in the blood. We analyzed the effect of NO-RBC reaction rate constant and blood flow velocity (thus, wall shear stress) on luminal and abluminal NO bioavailability, and nitrite and nitrate transport.

The 7 fold lower NO-RBC reaction rate constant increases only 1.3–4.3 fold NO bioavailability in vascular wall

It is now established that RBCs scavenge NO at a much slower rate than free Hb. Theoretical analyses combined with experimental studies involving NO-RBC interactions helped establish that the various diffusional resistances effectively reduce the amount of NO consumed by RBC-Hb (Azarov et al., 2005; Deonikar and Kavdia, 2009a; Deonikar and Kavdia, 2009b; Han and Liao, 2005; Lancaster, 1994; Liao et al., 1999; Liu et al., 1998; Liu et al., 2002; Vaughn et al., 2000). Recently, Deonikar et al (Deonikar and Kavdia, 2009b) reported a much lower NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ using a flow system device that simulates some of the in vivo hemodynamic flow conditions. This $k_{\text{NO-RBC}}$ is 7 fold lower than the NO-RBC reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ used by most mathematical models (Carlsen and Comroe, 1958). For both the arterioles, the NO levels in the cell free layer adjacent to vessel wall and the RBC rich core increased as the NO-RBC reaction rate constant decreased, indicating that lowering the NO-RBC reaction rate constant led to lower NO consumption by the RBCs and increase in the steady state NO concentrations in the vascular lumen. In addition, the results from this study predict that the lower $k_{\text{NO-RBC}}$ increases NO concentration in the

vascular wall. However at the endothelium and smooth muscle, the increase in the NO concentration for $k_{\text{NO-RBC}}$ of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ was 1.6–4.4 times the NO concentration for $k_{\text{NO-RBC}}$ of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$.

A number of studies have reported the vascular wall NO concentration range of 300–1000 nM in several different vascular beds (Bauser-Heaton and Bohlen, 2007; Buerk et al., 1996; Zhang et al., 1995). Recently, Bohlen et al. (Bohlen et al., 2009) reported arteriolar wall NO concentration in the range of 400–1000 nM using microelectrodes in 50–70 μm ID arterioles. In this study, the predicted NO concentration were 281–1163 nM in the smooth muscle cell layer for 50 μm ID arteriole over the blood velocity range of 0.5–4 cm/s for $k_{\text{NO-RBC}}$ of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. This validates the use of $k_{\text{NO-RBC}}$ of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for the NO transport models in the microcirculation.

Under right conditions downstream NO biotransport is possible

Mathematical modeling of NO biotransport in microcirculation has provided explanations on how NO is able escape the highly effective NO scavenger Hb present in large quantities in close proximity of site of NO production and perform its vasodilation function. However, the majority of mathematical models of NO transport in microcirculation have neglected the convective transport of NO along the blood vessels (Butler et al., 1998; El-Farra et al., 2003; Jeffers et al., 2006; Tsoukias, 2008; Tsoukias and Popel, 2002). The model developed in this study simulates both axial and radial NO biotransport in arterioles of 50 and 200 μm diameter. The results indicate that an accumulation of NO in the vascular lumen along the direction of the blood flow for both arterioles. The accumulation was significant for $k_{\text{NO-RBC}}$ of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ over a wide range of the blood velocities used. The results corroborate that under certain circumstances NO can transport from upstream to downstream as observed in the recent experimental findings in resistance vessels by Bohlen et al. (Bohlen et al., 2009).

For a given NO-RBC reaction rate constant, as the blood velocity increased, NO availability at endothelium and SMC layer increased linearly for both arterioles. This could be explained by the fact that increase in velocity would increase the wall shear stress leading to increased endothelial NO production. At $k_{\text{NO-RBC}}$ of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, the endothelium NO concentration was 32, 106, 198, and 750 nM for velocity of 0.1, 0.5, 1 and 4 cm/s, respectively for 50 μm arteriole. Thus, the change in NO concentration was –70, 87, and 608% for velocity of 0.1, 1 and 4 cm/s, respectively from the NO concentration at physiologic velocity of 0.5 cm/s (Kavdia and Popel, 2003; Vaughn et al., 1998b). At $k_{\text{NO-RBC}}$ of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, the endothelium NO concentration was 127, 241, 381, and 1229 nM for velocity of 0.1, 0.5, 1 and 4 cm/s, respectively for 50 μm arteriole. Thus, the change in NO concentration was –47, 59, and 412% for velocity of 0.1, 1 and 4 cm/s, respectively from the NO concentration at 0.5 cm/s velocity. Thus, the percentage change in the NO concentration for a change in the flow rate was smaller for the lower $k_{\text{NO-RBC}}$. The similar trend was observed in the larger arteriole.

Implications for physiologic NO transport

NO bioavailability preservation in microcirculation, especially under hypoxic conditions, has been a subject of intense investigation in recent years (Basu et al., 2007; Chen et al., 2008a; Chen et al., 2007a; Diesen et al., 2008; Gladwin et al., 2006; Jensen, 2009; Singel and Stamler, 2005; Stamler et al., 1997). Two products of NO metabolism in microcirculation, SNOHb and nitrite, have been attributed to preservation of NO bioactivity in the microcirculation. Formed in oxygenation cycle in the lungs, SNOHb is reported to transfer NO to low molecular weight thiols, facilitating NO transport to cause vasodilation (Diesen et al., 2008; Jia et al., 1996; Singel and Stamler, 2005; Stamler et al., 1997). On the other hand, deoxy-Hb reacts with nitrite to generate NO which is transported to the vascular wall in the form of NO or unreactive NO

species to effect vasodilation (Basu et al., 2007; Gladwin et al., 2006; Jensen, 2009; Nagababu et al., 2003). However, several theoretical analyses have shown that SNOHb or nitrite alone would not be able to produce enough NO to cause vasodilation. From our results, it is clear that there is a significant accumulation of NO (especially at NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) in the vascular lumen resulting from lower NO consumption in the lumen and convectional transport in the axial direction. The presence of NO in nanomolar levels would significantly increase the formation of SNOHb and lower the NO concentration gradient from endothelium to vascular lumen leaving more NO availability at the endothelium and vascular wall. The lack of steep NO concentration gradient due to presence of NO in the vascular lumen (which is caused by lower NO-RBC reaction rate constant and downstream NO transport) along with NO generation from either transnitrosation of SNOHb or nitrite reductase activity of deoxyHb could help explain NO bioactivity preservation in microcirculation.

Mixing cup NO, Nitrite and nitrate concentrations exiting the arteriolar segments

The mixing cup NO concentrations increased linearly with blood velocity and decreased with $k_{\text{NO-RBC}}$ as shown in Figure 7 (panels A and D). For a given $k_{\text{NO-RBC}}$ as the blood velocity increased, the residence time for NO reactions in the vascular lumen would decrease, leading to lower NO consumption and higher NO concentrations in the vascular lumen. The increase in mixing cup NO concentrations with decrease in $k_{\text{NO-RBC}}$ can be attributed to the reduced NO consumption by RBCs. Mixing cup NO concentrations were higher for 50 μm diameter arteriole than that of 200 μm diameter arteriole. The mixing cup NO concentrations ranged from ~ 3 –263 nM and ~ 0.1 –20 nM for 50 and 200 μm diameter arterioles, respectively. The reported NO concentrations are ~ 350 nM in arterioles of size of ~ 50 –70 μm diameter arterioles (Bohlen et al., 2009).

Nitrite is a product of NO autooxidation reaction. We estimated the total amount of nitrite formed in the vascular lumen of the arteriolar segments. As shown in Figure 7 (panels B and E), the nitrite formation increased with an increase in the velocity for both the arterioles at all $k_{\text{NO-RBC}}$'s, except for 0.1 cm/s velocity at $k_{\text{NO-RBC}} = 0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. The nitrite formation increased with a decrease in $k_{\text{NO-RBC}}$. These results can be interpreted as both decreasing the NO-RBC reaction rate constant or increasing the wall shear stress would lead to increasing NO availability, resulting in an increase in the nitrite formation. It is possible that our model underestimates the nitrite formation since the model only calculates the nitrite formed in the lumen of arteriolar segment and does not account entrainment of nitrite from the upstream vessel lumen and the vascular tissue. The nitrite concentrations estimated in this study ranged from 0.05–2.37 pM for 50 μm ID arteriole and 0.03–2.41 pM for 200 μm ID arteriole whereas the reported physiological plasma nitrite concentrations are in the range of 0.1–0.6 μM (Kleimbongard et al., 2003).

NO reacts with oxyHb in the RBCs to form metHb and nitrate. In this study, we predicted that the nitrate formations decreased with an increase in the velocity for all $k_{\text{NO-RBC}}$'s in both arteriole segments (see Figure 7 (panels C and F)). It is worth noting that despite the increase in the NO availability in the vascular lumen associated with the increase in velocity, the nitrate formation decreased with the increase in the velocity for a given $k_{\text{NO-RBC}}$. The possible explanation for this observation is that the NO availability would increase with an increase in the velocity because of increased shear stress dependent endothelium NO production but the residence time for NO-RBC reaction in the vascular lumen would decrease leading to decreased nitrate formation. The nitrate formations decreased with a decrease in $k_{\text{NO-RBC}}$ for a given blood velocity for both arterioles. The nitrate formation was in the range of ~ 1.03 –5.9 μM for 50 μm ID arteriole and ~ 1.31 –4.25 μM for 200 μm ID arteriole. In vivo plasma nitrate concentrations of 32.8 ± 10 μM were reported by Dreissigacker et al. (Dreissigacker et al.,

2010). Perfusion of isolated vessel with blood and measurement of nitrate concentration can be performed to validate the prediction of nitrate formation.

In conclusion, a cylindrical geometry mathematical model that incorporates radial and axial transport of NO in arterioles to explore the possibility of downstream transport of NO in the blood was developed. The effect of NO-RBC reaction rate constant and blood flow velocity (thus, wall shear stress) on luminal and abluminal NO bioavailability, and nitrite and nitrate transport were analyzed. Results predict that the NO concentration can increase to 281–1163 nM for $k_{\text{NO-RBC}}$ of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and are similar to the experimentally measured vascular wall NO concentration range of 300–1000 nM in several different vascular beds. The results also indicate that the downstream transport of NO is possible under right circumstances.

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Figure 1b

Figure 1.

a) Schematic diagram of model geometry. The model geometry consists of concentric cylinders representing subregions of luminal and abluminal regions of the arteriole. The arteriole is divided into luminal and abluminal regions. Luminal region contains subregions representing RBC rich core and cell free zone (CFZ). Abluminal regions include endothelium (EN), interstitial space (IS), smooth muscle cell layer (SMC), non perfused tissue (NPT) and parenchymal tissue (PT) perfused with capillaries. NO released from endothelial cells in shear stress dependant manner diffuses to vascular lumen and smooth muscle cells; b) Mesh grid representing the finite elements. The software generates finite elements in proportion with the concentration gradients in the different regions. More finite elements are generated where the concentration gradient is higher. Relative accuracy was set to be 0.005 for all the simulations.

Figure 2.

NO concentration profiles across all the regions of arterioles at the end of the arteriolar segments. NO concentrations at the end of the arteriolar segments is plotted as a function of distance from the vessel center for 50 μm ID arteriole (panels A–C) and 200 μm ID arteriole (panels D–F). For both the arterioles, NO concentration in all the subregions increased with blood velocity for all the NO-RBC reaction rate constants.

Figure 3.

A) NO concentration distribution in vascular lumen of arteriole segment of 50 μm ID and 500 μm length. Panels A–D represent the NO concentration for NO-RBC reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for blood velocity range of 0.1–4 cm/s, respectively. Panels E–H represent the NO concentration for NO-RBC reaction rate constant of $0.5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for blood velocity range of 0.1–4 cm/s, respectively. Panels I–L represent the NO concentration distribution for NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for blood velocity range of 0.1–4 cm/s, respectively. NO availability in the vascular lumen increased with decrease in NO-RBC reaction rate constant and increase in blood velocity. B) NO concentration in part of vascular lumen (25 μm from the vessel wall) of arteriolar segment of 200 μm ID and 2000 μm length. Panels A–D represent the NO concentration for NO-RBC reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for blood velocity range of 0.1–4 cm/s, respectively. Panels E–H represent the NO concentration for NO-RBC reaction rate constant of $0.5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for blood velocity range of 0.1–4 cm/s, respectively. Panels I–L represent the NO concentration for NO-RBC reaction rate constant of $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for blood velocity range of 0.1–4 cm/s, respectively. NO availability in the vascular lumen increased with decrease in NO-RBC reaction rate constant and increase in blood velocity.

Figure 4.

A) Nitrite concentration profiles in vascular lumen of arteriole segment of 50 μm ID and 500 μm length. Panels A–D, E–H and I–L represent the nitrite concentration profiles for NO-RBC reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, $0.5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively for blood velocity range of 0.1–4 cm/s. Nitrite concentrations in the vascular lumen increased with decrease in NO-RBC reaction rate constant and increase in blood velocity. B) Nitrite concentration in part of the vascular lumen (25 μm from the vessel wall) of arteriolar segment of 200 μm ID and 2000 μm length. Panels A–D, E–H and I–L represent the nitrite concentration profiles for NO-RBC reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, $0.5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively for blood velocity range of 0.1–4 cm/s. Nitrite concentrations in the vascular lumen increased with decrease in NO-RBC reaction rate constant and increase in blood velocity.

Figure 5.

A) Nitrate concentration profiles in RBC core region of vascular lumen of arteriole segment of 50 μm ID and 500 μm length. Panels A–D, E–H and I–L represent the nitrate concentration profiles for NO-RBC reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, $0.5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively for blood velocity range of 0.1–4 cm/s. Nitrate concentrations in the vascular lumen increased with decrease in NO-RBC reaction rate constant and increase in blood velocity. B) Nitrate concentration in part of the RBC core region vascular lumen (from RBC core-CFZ interface to 25 μm distance from the vessel wall) of 200 μm diameter and 2000 μm length arteriole segment. Panels A–D, E–H and I–L represent the nitrate concentration profiles for NO-RBC reaction rate constant of $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, $0.5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively for blood velocity range of 0.1–4 cm/s. Nitrate concentrations in the vascular lumen decreased with decrease in NO-RBC reaction rate constant and increase in blood velocity.

Figure 6.

NO concentration levels at endothelium and smooth muscle cell layer at the end of arteriolar segments. NO concentration levels for 50 μm ID arteriole at the endothelium (A) and smooth muscle cell layer (B) are plotted as a function of blood velocity. Panels C and D represent the endothelium and smooth muscle cell layer NO concentration levels, respectively, for 200 μm ID arteriole. $C_{\text{NO}}^{\text{en}}$ represents the NO concentrations at the cell free zone-endothelium interface and $C_{\text{NO}}^{\text{SM}}$ represents NO concentrations at the smooth muscle cell-non perfused tissue interface.

Figure 7.

Mixing cup concentrations of NO, nitrite and nitrate exiting the arteriolar segments. NO, nitrite, and nitrate are plotted as a function of blood velocity and k_{NO-RBC} . Panels A, B and C represent NO, nitrite and nitrate concentrations in the exiting perfusate of 50 μm ID arteriolar segment, respectively. Total NO, nitrite and nitrate mixing cup concentrations for 200 μm ID arteriolar are plotted in panels D, E and F respectively. Mixing cup NO concentrations increased linearly with blood velocity and increased with decrease in NO-RBC reaction rate constants for both arterioles. Total nitrite formations increased with increase in blood velocity and decrease in NO-RBC reaction rate constant for both the arterioles. Total nitrate formations decreased with blood velocity, and increased with increase in NO-RBC reaction rate constant.

Table 1

Model parameters

Parameter	Description	Value
D_{NO}	NO diffusivity	$3.3 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Malinski et al., 1993)
$P_{NO_control}$	Arteriolar endothelial NO release rate	$5.3 \times 10^{-12} \text{ moles cm}^{-2} \text{ s}^{-1}$ (Vaughn et al., 1998b)
q_{NO_cap}	Capillary endothelial NO production rate	$8.6 \times 10^{-7} \text{ M s}^{-1}$
k_{NO-RBC}	NO-RBC reaction rate constant	$0.2 \times 10^5, 0.5 \times 10^5, 1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (Carlsen and Comroe, 1958; Deonikar and Kavdia, 2009b)
k_{NO-O_2}	NO autooxidation rate constant	$9.6 \times 10^6 \text{ M}^{-2} \text{ s}^{-1}$ (Lewis and Deen, 1994)
k_{sGC-NO}	NO reaction rate constant with sGC	$5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ (Vaughn et al., 1998b)
k_{cap}	NO-RBC reaction rate in capillaries	12.4, 7.11, 1.77 s^{-1}
C_{RBC-Hb}	RBC hemoglobin concentration at 45% hematocrit	9.135 mM
C_{O_2}	Oxygen concentration	27 μM
v_{zmax}	Blood velocity	0.1, 0.5, 1, 4 cm s^{-1}