Analysis of nitric oxide donor effectiveness in resistance vessels

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Submitted 24 September 2004; accepted in final form 8 January 2005

Hyduke, Daniel R., and James C. Liao. Analysis of nitric oxide donor effectiveness in resistance vessels. Am J Physiol Heart Circ Physiol 288: H2390–H2399, 2005. First published January 14, 2005; doi:10.1152/ajpheart.00990.2004.—Decreased nitric oxide (NO) bioavailability is associated with a number of pathological conditions. Administration of a supplemental source of NO can counter the pathological effects arising from decreased NO bioavailability. A class of NO-nucleophile adducts that spontaneously release NO (NONOates) has been developed, and its members show promise as therapeutic sources of NO. Because the NONOates release NO spontaneously, a significant portion of the NO may be consumed by the myriad of NO reactive species present in the body. Here we develop a model to analyze the efficacy of NO delivery, by membrane-impermeable NONOates, in the resistance arterioles. Our model identifies three features of blood vessels that will enhance NONOate efficacy: 1) the amount of NO delivered to the abluminal region increases with lumen radius; 2) the presence of a flow-induced red blood cell-free zone will augment NO delivery; and 3) extravasation of the NONOate into the interstitial space will increase abluminal NO delivery. These results suggest that NONOates may be more effective in larger vessels and that NONOate efficacy can be altered by modifying permeability to the interstitial space.

Vasodilation; NONOate; microcirculation; extravasation

NITRIC OXIDE (NO) is a versatile biological messenger that plays roles in a number of physiological and pathological processes. NO is produced in a variety of cell types by the enzymatic conversion of L-arginine to L-citrulline. In the cardiovascular system, endothelium-derived NO is involved in a number of regulatory functions. NO produced by endothelial nitric oxide synthase (eNOS) has been documented to play a part in vasodilation, platelet adhesion and aggregation, leukocyte adhesion to the endothelium, and control of vascular smooth muscle cell proliferation (19).

Decreased bioavailability of endothelium-derived NO is associated with a number of pathological conditions (19). In particular, decreased levels of NO have been observed in ischemia-reperfusion injury (12), hypertension and hypercholesterolemia (34), atherosclerosis (32, 33), and sickle cell disease (35). Clinically available sources of NO include sodium nitroprusside, organic nitrates, inhaled NO gas, and L-arginine.

Organic nitrates, such as nitroglycerin, have been used for over 100 years to treat ischemic heart disease (2). NO is enzymatically produced from nitroglycerin by mitochondrial aldehyde dehydrogenase (8) and other sulfur-dependent mechanisms (14). Nitroglycerin vasodilation efficacy is highest in capacitance veins and conductive arteries (2). Chronic nitroglycerin therapy leads to tolerance problems, which are thought to arise from inhibition of the nitrate reductase activity of mitochondrial aldehyde dehydrogenase (8). Additionally, there is some concern that long-term nitroglycerin therapy increases morbidity (31).

Sodium nitroprusside (SNP) has been used to reduce hypertension in humans since 1928 (13). SNP is a potent vasodilator that is used to treat severe hypertension and heart failure and for controlled intraoperative hypotension. In vivo, NO release from SNP is enhanced via reaction with a membrane-bound protein (21). The biological half-life of SNP is 1–2 min (13). In the process of NO production, SNP releases cyanide anions, which may have toxic effects (36).

A relatively new class of NO-releasing molecules, known as the NONOates, is being investigated as a therapeutic source of NO. NONOates are nucleophile-NO complexes that have the basic form XNONO, where X represents the nucleophile group (10, 17, 27, 30). The NONOates spontaneously release NO in a wide variety of biological and experimental solutions. A number of the properties of the NONOates, such as NO-release rate and membrane permeability, are a function of the nucleophile group. Because it is possible to design NONOates with desirable properties, the NONOates have the potential to supplement, or supplant, current forms of NO therapy. Diethylentriamine NONOate (DETA-NONOate) was found to be a selective pulmonary vasodilator in patients with acute respiratory distress syndrome (22). In addition, intravenous administration of a variety of NONOates has been shown to result in vasodilation in a feline model (3).

A number of NONOates with polar nucleophile groups were able to elicit a vasodilatory response (3). Because the polar nucleophile group hinders passive diffusion of the NONOate across lipid membranes, the NONOate will not be able to cross the smooth muscle cell membrane and hence will release NO in the extracellular regions. To activate soluble guanylate cyclase (sGC), the NO will need to diffuse from the extracellular space to the smooth muscle cytosol; however, there are a number of NO-reactive species that may prevent NO from reaching the smooth muscle. The most notable NO-reactive species in the circulatory system is hemoglobin (Hb). NO reacts with ferrous forms of Hb, either oxygenated (HbO2) or deoxygenated (deoxyHb), Hb, at near-diffusion-limited rates (−10^2−10^5 M^−1 s^−1) (7, 16). NO reacts with HbO2 to form oxidized Hb (metHb) and bio inert nitrate. NO binds to deoxyHb and forms nitrosylHb (HbNO), which does not have any known vasodilatory properties in normoxic conditions; however, a number of studies have indicated that HbNO may act as a reservoir of NO bioactivity when the oxygen concentration is reduced (39).
A number of theoretical studies of NO transport in microvessels have indicated that the high concentrations of HbO2 in blood will consume a large portion of the endothelium-derived NO (4, 6, 23, 46). The majority of these studies neglected the particulate nature of blood and treated blood as a continuum. Experimental and theoretical studies of NO transport in microvessels have indicated that compartmentalization of HbO2 inside of red blood cells (RBCs) leads to a reduction in the rate of NO consumption by blood (24, 25, 45). Using an isolated porcine artery, Liao’s group (24) has illustrated that flowing RBCs consume less endothelium-derived NO than stagnant RBCs. The reduction in NO consumption by flowing RBCs is attributed to a flow-induced RBC-free zone that forms near the microvessel walls. In addition, multiple reports have shown that a suspension of RBCs consumes NO 600–1,000 times slower than a solution of free Hb (25, 44, 45). Because the rate of NO uptake by RBCs can be modulated by treating RBCs with NO (15) and other chemicals (18) that modify the structure of proteins associated with the RBC membrane and cytoskeleton, we suggested that the RBCs possess an intrinsic barrier to NO diffusion. The Lancaster group (25) attributed the decrease in NO consumption to the presence of an extracellular boundary layer surrounding the RBCs.

In this work, we develop a model of NO delivery by membrane-impermeable NONOates in resistance arterioles. During model development, we investigated the feasibility of approximating the RBCs in the lumen as discrete RBCs in two-dimensional (2-D) and three-dimensional (3-D) configurations or in one-dimensional (1-D) configuration as an Hb-rich continuum. We began with the most realistic 3-D multicellular RBC model, which is then simplified to a more economical 2-D model without losing accuracy. We use our model to assess the impact that various physical characteristics of microvessels have on NO delivery by NONOates. In particular, we examine the impact of lumen radius, RBC-free zone thickness, and donor extravasation on NONOate efficacy.

**METHODS**

**Model geometry.** Finite element models of the vessel were composed of five regions (Fig. 1A): 1) the lumen (lu), 2) the endothelium (ec), 3) the interstitial space (is), 4) the smooth muscle (smc), and 5) the perivascular tissue (pt). In the 1-D model, the lumen consists of two regions: a cell-free zone (cfz) near the endothelium and an Hb-rich core (core) that approximates the RBC-rich center of the lumen (Fig. 1B). In the 2-D and 3-D models, the lumen is populated by discrete RBCs that are modeled as circles or spheres. The discrete RBCs are composed of two regions: an Hb-rich cytosol (cyt) and a thin NO-barrier layer (bar) that approximates the RBC membrane and the submembrane resistance to NO transport (Fig. 1C).

**Assumptions.** To investigate NO delivery by spontaneous donors of NO while maintaining a computationally tractable system, we introduce a number of simplifying assumptions: 1) Transport by convection is neglected. 2) All reactants, except NO, have constant concentrations. 3) All regions have homogeneous properties. 4) NO freely diffuses across the interfaces between the regions. 5) Far from the vessel wall, NO concentration does not change with distance. 6) The NO donor is membrane impermeable and is only present in the blood.

**Fig. 1.** Schematic for microvessel model of nitric oxide (NO) delivery by NONOates. A: blood vessel is composed of the lumen (lu), the endothelium (ec), the interstitial space (is), the smooth muscle (smc), and perivascular tissue (pt). B: in the one-dimensional (1-D) model, the lumen is composed of an hemoglobin-rich (Hb) core (core) that approximates the red blood cells (RBCs) and a cell-free zone (cfz) that approximates the glyocalyx and flow-induced cell free layer near the endothelium. C: in the two-(2-D) and three-dimensional (3-D) models, the RBCs are modeled as discrete cells composed of two layers: the Hb-rich RBC cytosol (cyt) and the barrier to NO diffusion (bar). Schematics are not drawn to scale. D: comparison of the the total amount of RBC surface area for three different spherical approximations of the erythrocytes: spheres with single RBC equivalent surface area-to-volume ratios (SA/V) (solid line), spheres with single RBC equivalent volumes (dashed line), and spheres with single RBC equivalent surface area (dotted line). Spheres with RBC equivalent SA/V maintain the correct amount of total surface area as a function of Hct. Total RBC surface area was calculated for a 100-μm long vessel with a 100-μm luminal radius.
plasma and interstitial space. 7) The NO donor is the only source of NO. 8) Autooxidation of NO is neglected. The autooxidation reaction is second order in NO, and in this study it should have a negligible impact on NO delivery. In preliminary studies, we found that autooxidation of NO had no discernible impact on NO delivery. 9) Only sGC-dependent vasodilation is considered.

**Governing equations.** The concentration of a chemical species as a function of time and space are described using a general mass balance:

$$\frac{\partial C_{i,j}}{\partial t} = D_{i,j} \nabla^2 C_{i,j} - \nabla \cdot J_{i,j} + R_{i,j}$$

(1)

where \(i\) is the species of interest, \(j\) is the region of interest, \(\nabla\) is the vector gradient operator, \(D_{i,j}\) is the Laplacian operator, \(C_{i,j}\) is the concentration of species \(i\) in region \(j\), \(D_{i,j}\) is the diffusion coefficient of species \(i\) in region \(j\), \(v_j\) is the velocity vector, and \(R_{i,j}\) represents all production and consumption terms for species \(i\) in region \(j\).

In the case of NO, we neglect convection, \(\nabla C_{NO,j} \cdot v_j = 0\), and apply the steady-state approximation for all regions to reduce the mass balance Eq. 1 to:

$$D_{NO,j} \nabla^2 C_{NO,j} + R_{NO,j} = 0$$

(2)

Substitution of the corresponding terms for diffusion coefficient of NO in region \(j\) (\(D_{NO,j}\)) and \(R_{NO,j}\) in each region of the models (Fig. 1) into Eq. 2 results in a set of partial differential equations describing the system.

In the three models considered, the reaction and diffusion terms are equivalent in the abluminal regions. Inside of the lumen, the 2-D and 3-D models have three distinct regions, the cytosol, the RBC, and pl, whereas the 1-D model is composed of two subregions, the core and the cfz. Parameters for each region are summarized in Table 1.

**Endothelium.** Because we are modeling NO-deficient microvessels and we neglect autooxidation of NO, the reaction term in the endothelium is 0.

$$R_{NO,ec} = 0$$

(3)

For a microvessel with a luminal radius \(r_{no}\) of 15 \(\mu\)m, the thickness of the endothelium \(d_{ec,rt}=15\) \(\mu\)m is set to 0.75 \(\mu\)m. Diffusion coefficient of NO inside endothelial cells \((D_{NO,ec})\) is set to \(3.3 \times 10^{-9}\) \(m^2/s\) based on an estimate of NO diffusion in tissue (47).

**Interstitial space.** In the interstitial space, NO can be produced by extravasating donor:

$$R_{NO,is} = n_{donor} \cdot k_{donor} \cdot C_{donor,is} = Q_{donor,is}$$

(4)

where \(n_{donor}\) is the number of molecules of NO produced per molecule of NO donor, \(k_{donor}\) is the NO release rate, and \(C_{donor,is}\) is the NO donor concentration. Because we assume that the NO donor concentration is constant, we can represent NO production from the NO donor as an approximate volumetric rate \((Q_{donor,pl})\) for all microvessels, the thickness of the interstitial space \(d_{interstitial}=0.75\) \(\mu\)m is set to 1.5 \(\mu\)m. In all simulations, the thickness of the perivascular region was 4,000 \(\mu\)m.

**Blood plasma.** In the blood plasma, we assume that NO is produced by the donor in a concentration-dependent fashion:

$$R_{NO,pl} = n_{donor} \cdot k_{donor} \cdot C_{donor,pl} = Q_{donor,pl}$$

(5)

where \(n_{donor}\) is the number of molecules of NO produced per molecule of NO donor, \(k_{donor}\) is the NO release rate, and \(C_{donor,pl}\) is the NO donor concentration. Because we assume that the NO donor concentration is constant, we can represent NO production from the NO donor as an approximate volumetric rate \((Q_{donor,pl})\). Diffusion coefficient of NO inside plasma \((D_{NO,pl})\) is set to \(3.3 \times 10^{-9}\) \(m^2/s\) based on an estimate of NO diffusion in water (26).

**Cell-free zone.** In all of the models, there is an erythrocyte-free layer of plasma adjacent to the endothelium. This zone represents both the glycocalyx (48) and the flow-induced cell-free zone (24, 38). The glycocalyx thickness has been measured as \(-0.5\) \(\mu\)m in capillaries (48) and \(-2.5\) \(\mu\)m in a microvessel with a 75- \(\mu\)m radius (43). To approximate the glycocalyx, the minimum thickness for this zone \(d_{cfz}\) is set to 1.5 \(\mu\)m. We assume that the thickness of the flow-induced RBC-free zone on NONOate efficacy, we use cell-free zone thicknesses extracted from Sharan and Popel (38).

**RBCs.** RBCs are approximated as circles or spheres composed of two distinct regions: the barrier to NO diffusion (bar) and the cytosol (cyt). RBC membrane-associated barrier. Because the barrier to NO diffusion is composed of relatively NO inert compounds (18) and we neglect autooxidation of NO, there is no production or consumption of NO in the barrier region:

$$R_{NO,bar} = 0$$

(7)

The value of the NO diffusion coefficient in the RBC NO barrier is computed from the RBC permeability to NO, using film theory approximation:

$$\frac{D_{NO,bar}}{d_{bar}} \approx P_{RBC}$$

(8)

where \(D_{NO,bar}\) is the effective NO diffusion coefficient in the RBC NO-barrier region, \(d_{bar}\) is the barrier thickness, and \(P_{RBC}\) is the permeability of the RBC to NO. We use an RBC permeability of \(4.15 \times 10^{-4}\) \(m^2/s\) (44).

**RBC cytosol.** For the RBC cytosol, NO is assumed to react solely with HbO2. Because the concentration of HbO2 is much greater than the concentration of NO, we assume that the concentration of HbO2 is constant:

$$R_{NO, cyt} = -k_{NO-HbO2} \cdot C_{HbO2,cyt} \cdot C_{NO, cyt} = -k_{RBC} \cdot C_{NO, cyt}$$

(9)

where \(k_{NO-HbO2}\) is the rate of NO dioxygenation by HbO2, \(C_{HbO2,cyt}\) is the concentration of HbO2 in the RBC cytosol, and \(k_{RBC}\) is the pseudo-first-order reaction constant.
RBC radius. Because of the computational complexity associated with modeling the biconcave shape of RBCs, we elected to approximate RBCs as circles or spheres. In the physical system that we are investigating, the most important characteristics of the RBCs are the total RBC surface area and, to a lesser extent, the total RBC volume. To maintain the correct amount of RBC surface area and volume in our simulations, regardless of model dimension, lumen radius, or hematocrit (Hct), we approximated RBCs with circles or spheres that possess the same surface area-to-volume ratio as an human erythrocyte (RBCs \(\text{SA/V} = 1.42 \text{ mm}^{-1}\)). The radii for the circle and the sphere are 1.4 and 2.1 mm, respectively. In Fig. 1D, we plot total RBC surface area versus Hct for a 100-µm long vessel with a 100-µm radius. When the RBCs are approximated by spheres that possess the same volume (Fig. 1D, dashed lines) or surface area (Fig. 1D, dotted lines), as an RBC results in a significant deficit in the total RBC surface area and hence will underestimate the diffusion of NO into the RBCs.

Hb-rich core. In the 1-D simulation, we neglect the particular nature of blood and approximate the RBC-rich core as a continuum (core). In this region, NO can be consumed by reaction with HbO2 and produced from the NO donor:

\[
R_{\text{NO, core}} = -k_{\text{NO-HbO2, core}} C_{\text{HbO2, core}} C_{\text{NO, core}} + Q_{\text{donor, core}} \tag{10}
\]

where \(k_{\text{NO-HbO2, core}}\) is the rate of NO dioxygenation by HbO2 in the core. Because the Hb-rich core is a mixture of RBC cytosol, RBC barrier, and blood plasma, each of the parameters must be scaled. The concentration of HbO2 in this region is calculated by multiplying the core hematocrit (Hct\(_{\text{core}}\)) by the concentration of Hb in an erythrocyte:

\[
C_{\text{HbO2, core}} = C_{\text{HbO2, cyt}} \text{Hct}\(_{\text{core}}\) \tag{11}
\]

where Hct\(_{\text{core}}\) is calculated by multiplying the lumen Hct by the lumen volume (\(V_{\text{lp}}\)) and dividing by the core volume (\(V_{\text{core}}\)):

\[
\text{Hct}\(_{\text{core}}\) = \frac{\text{Hct} \times V_{\text{lp}}}{V_{\text{core}}} \tag{12}
\]

The NO production rate is calculated by scaling the blood plasma production rate:

\[
Q_{\text{donor, core}} = Q_{\text{donor, pl}} \frac{V_{\text{lp, core}}}{V_{\text{core}}} \tag{13}
\]

where \(V_{\text{lp, core}}\) is the volume of plasma in the core. \(D_{\text{NO, pl}}\) is approximated as a linear combination of \(D_{\text{NO, pl}}\) and \(D_{\text{NO, cyt}}\):

\[
D_{\text{NO, core}} = (1 - \text{Hct}\(_{\text{core}}\)) D_{\text{NO, pl}} + \text{Hct}\(_{\text{core}}\) D_{\text{NO, cyt}} \tag{14}
\]

Boundary conditions. In this system, NO is assumed to diffuse freely across the interfaces between regions, which leads to the following boundary condition:

\[
D_{\text{NO}} \frac{\partial C_{\text{NO, +}}}{\partial n} = D_{\text{NO}} \frac{\partial C_{\text{NO, -}}}{\partial n} \tag{15}
\]

where \(\vec{n}\) is the vector normal to the boundary and the subscript plus sign indicates one side of an interface and the subscript minus sign indicates the opposing side. Far from the vessel wall the NO concentration is assumed to be constant:

\[
D_{\text{NO}} \frac{\partial C_{\text{NO}}}{\partial r} = 0 \tag{16}
\]

where \(r\) is the radial distance from the vessel center.

Numerical methods. The system of differential equations was solved using finite element simulation packages. The 1-D and 2-D models were solved using Femlab 3.0a (Comsol, Stockholm, Sweden). The 3-D model was solved using CFD-ACE+ 2003 (ESI Group, Paris, France). For all three models, the mesh was successively refined until changes in the number of mesh elements had no significant influence on simulation results. For the outer boundary condition (Eq. 16), a radius of \(-4,000 \mu m\) was employed; for the microvessels investigated, this outer radius was large enough to ensure that the results were not appreciably influenced by the location of the outer boundary condition.

RESULTS

With the appropriate scaling factor, the simplified 2-D model is a viable approximation of the 3-D model. Because of the complex topology of the microvasculature, it is difficult to compose, and computationally expensive to simulate, a model that is an exact replica of a microvessel. To develop a computationally tractable model of microvessels, we approximate the vessel wall as a series of concentric cylinders and the RBCs as spheres (3-D) or circles (2-D). In the 3-D and 2-D models, the spheres and circles need to be designed to capture the physical characteristics of the RBCs that are essential to the system that is being investigated. Because the majority of the reaction between NO and HbO2 in the RBC occurs near the RBC surface, we chose to approximate the RBCs with spheres and circles that possess the same surface area-to-volume ratio (SA/V) as an human erythrocyte. The use of the SA/V as the shape constraint leads to the correct amount of surface area regardless of vessel radius.

Although it is possible to develop a 3-D NO transport model, with RBCs approximated by spheres, the computational costs associated with the model limit the use of the model. Therefore, it is desirable to simplify the 3-D model to lower dimensions while maintaining the key features. When the SA/V constraint is employed, there is little difference between the 2-D and 3-D simulation results (Fig. 2A). When we remove the SA/V constraint and use circles and spheres with equivalent radii (\(R_{\text{RBC}} = 2.04 \mu m\)), there is a significant difference between the simulated concentration profiles (Fig. 2B). Because the SA/V constraint leads to close agreement in the 2-D and 3-D predictions, it is not necessary to employ the computationally expensive 3-D model in this work.

The 1-D continuum model is characteristically different from the 2-D discrete RBC model. To determine whether we could further simplify the microvessel model, we investigated the possibility of employing a 1-D model. In the 1-D model, the RBCs cannot be discrete particles; instead, they are approximated by an Hb-rich core (Fig. 1B). To approximate the diffusional resistances to NO reaction with RBC HbO2, a reduced NO oxidation rate by HbO2 (\(k_{\text{NO-HbO2, core}}\)) must be employed for the 1-D case. In Fig. 2C, we present the predicted NO concentration profiles for the 2-D discrete RBC model and for the 1-D Hb-rich core model with two different values of \(k_{\text{NO-HbO2, core}}\). The values of \(k_{\text{NO-HbO2, core}}\) were selected in an attempt to fit the 1-D model predictions to the 2-D model prediction in the luminal and abluminal regions. Figure 2C shows that it is not possible to fit the 1-D predictions to the 2-D predictions using a single value of \(k_{\text{NO-HbO2, core}}\). This discrepancy is due to the 1-D assumption that the NONOate and the HbO2 are homogeneously mixed within the core.

In a recent paper, Tsoukias and Popel (42) formulated a 1-D model to predict abluminal NO concentrations of capillaries. In this model, they used a discrete RBC model to estimate a 1-D \(k_{\text{NO-HbO2, core}}\) required to realize an abluminal NO concentration similar to the discrete RBC model. For a 15-µm radius microvessel, we were able to match the 1-D...
The model is insensitive to small variations in $k_{\text{NO-HbO}_2}$ and $D_{\text{NO,CP}}$. The values of the NO reaction rate with Hb ($k_{\text{NO-HbO}_2}$) and the diffusion coefficient of NO inside of RBCs ($D_{\text{NO,RBC}}$) have not been accurately measured at 37°C. To estimate the impact of variations in these parameters, we examined the impact of variations in these parameters on the simulation output. We performed simulations using values of $k_{\text{NO-HbO}_2}$ that were estimated at 20°C (0.30 × 10^{-5} M^{-1}s^{-1} and 0.89 × 10^{-5} M^{-1}s^{-1}) (11, 16) and a value that was extrapolated for 37°C (1.6 × 10^{-5} M^{-1}s^{-1}) (41). $D_{\text{NO,RBC}}$ was varied from an estimated value (0.88 × 10^{-9} m^2/s) (44) to the diffusion coefficient of NO in plasma (3.3 × 10^{-9} m^2/s) (26). Such variations in $k_{\text{NO-HbO}_2}$ and $D_{\text{NO,RBC}}$ did not change the predicted NO profiles by more than a fraction of a percent (results not shown).

Estimation of the NO production rate required to modulate sGC activity in resistance arterioles. The resistance arterioles are thought to be the primary site of controlling systemic blood pressure. The radii of these arterioles range from ~10 to 100 μm (49). In order for a NONOate to reduce systemic blood pressure, it must deliver enough NO to activate sGC to the smooth muscle. Condorelli and George (9) have estimated that at least 5 nM of NO is required to modulate sGC activity.

In Fig. 3A, we show the NO concentration profiles for a number of NO production rates in a vessel with a 15-μm radius. Our model indicates that an NO production rate greater than 1 μM/s is required to raise the mean NO concentration ($C_{\text{NO,smc}}$) in the smooth muscle cell region above 5 nM. Hereafter, $Q_{\text{donor-sGC}}$ will be used to refer the minimum $Q_{\text{donor}}$ required to raise $C_{\text{NO,smc}}$ above 5 nM. For a donor, such as spermine NONOate, with $n_{\text{donor}} = 2$ and a half-life ($t_{1/2}$) of 39 min (20), a steady-state concentration of ~1.7 mM will be required to maintain a NO production rate of 1 μM/s (Eq. 17).

\[
C_{\text{donor-sGC}} = \frac{Q_{\text{donor-sGC}} \ln(2)}{n_{\text{donor}} t_{1/2}} \tag{17}
\]

To determine the impact of the vessel radius on abluminal delivery of NO, we calculated $C_{\text{NO,smc}}$ for microvessels with...
radii ranging from 15 to 50 μm (Fig. 3B). For a constant NO production rate (1.54 μM/s), $C_{\text{NO,smc}}$ increases with increasing vessel radius.

**Flow-induced increase in RBC-free zone increases the ability of NONOates to deliver NO to the smooth muscle.** In microvessels, fluid flow causes RBCs to aggregate in the center of the vessel lumen. This aggregation leads to the presence of an RBC-free zone near the endothelium glycocalyx. The combined thickness of the RBC-free zone and glycocalyx has been observed to be as high as 4 μm. The size of the cell-free zone ($\delta_{\text{cfz}}$) varies in a nonlinear fashion with vessel diameter (38).

To examine the impact of the flow-induced RBC-free zone ($\delta_{\text{cfz}}$) on NONOate efficacy, we used cell-free zone thickness estimates that correspond to a 45% Hct from Sharan and Popel (38). Incorporation of the flow-induced RBC-free zone thickness estimates of Sharan and Popel effectively doubles the abluminal concentration of NO for a 15-μm radius microvessel (Fig. 4C). A NO production rate of 0.57 μM/s is required to produce sufficient NO in the smooth muscle region (dashed line) to exceed the minimum threshold for modulating sGC activity (dotted line). Reference case parameters were used unless otherwise specified.

**Extravasation of NO donor into interstitial space.** The walls of many microvessels contain a number of pores that allow small hydrophilic molecules (~20–300 Å) to pass from the lumen to interstitial space (28). Diffusion of membrane-impermeable NO donors into the interstitial space should enhance abluminal delivery of NO. To simulate the impact of extravasation on NONOate efficacy, we added an NO production term to the interstitial space (Eq. 4). To retain a basis for comparison between the extravasation and no extravasation scenarios, for a given lumen radius, the total number of moles of NO released...
per second was the same in the two scenarios. In this set of simulations, we employed the variable cell-free zone-thickness estimates from Sharan and Popel (38). Figure 5A illustrates that extravasation of NO donor into the interstitial region increases the abluminal NO concentration. Extravasation increases $C_{NO,amc}$ by $\sim 10\%$ for a $r_{lu} = 15 \mu m$ to $\sim 20\%$ for $r_{lu} = 50 \mu m$ (Fig. 5B). For a 15-\mu m radius microvessel with $\delta_{cfz} = 3.5 \mu m$, extravasation of NO donor reduces $Q_{donor-sGC}$ to 0.51 $\mu M/s$ (Fig. 5C).

**DISCUSSION**

The illustration of the spontaneous release of NO from a number of NO-nucleophile complexes (NONOates) by Maragos et al. (27) has led to the development of NO donors that are beneficial to NO research and potentially beneficial in a clinical setting. Because a number of properties of the NONOate, such as NO release rate and membrane permeability, are a function of the nucleophile, it is possible to design a NONOate with desired properties by selecting an appropriate nucleophile. Here we develop a theoretical model of NO delivery by membrane-impermeable NONOates in microvessels. With this model, we identify characteristics of the microvessels that will influence NO delivery.

*Model development.* Because of the complex topology of the microvasculature, it is difficult to compose, and computationally expensive to simulate, a model that is an exact replica of a microvessel. To develop a computationally tractable microvessel model, we created 3-D, 2-D, and 1-D approximations of microvessels. In these approximations, the lumen and surrounding tissue are modeled as a series of cylinders (Fig. 1A). In the lumen, the RBCs were approximated by spheres, circles, and an Hb-rich core for the 3-D, 2-D, and 1-D models, respectively.

When a population of RBCs with spheres, or circles, in a stagnant system are approximated, care must be taken in selecting the size of the spheres. Because it is impossible to design a sphere or circle that maintains identity to an erythrocyte in both volume and surface area, additional aspects of the system must be considered. In the system we are modeling, we need to consider the total volume occupied by the RBCs ($V_{RBCs}$) and the total RBC surface area ($S_{ARBCs}$). Maintaining the correct $V_{RBCs}$ is important because it dictates the amount of plasma in the lumen ergo the amount of NONOate. The $S_{ARBCs}$ limits the rate of NO entry into the RBCs. Because the reaction of NO with intracellular Hb is much faster than NO entry, it is important to incorporate the correct $S_{ARBCs}$ into the model. To maintain $V_{RBCs}$ and $S_{ARBCs}$, we approximate the population of RBCs by a population of spheres or circles that possess the same SA/V as an erythrocyte (SA/VRBC). Approximating RBCs by spheres or circles that possess the same volume or surface area as an erythrocyte results in a deficit in the total RBC surface area (Fig. 1D). Because the magnitude of this deficit increases with Hct, its impact may be negligible at low (<1) Hct.

Although the 3-D model is the best approximation of the system of interest, the computational cost of the simulations makes the model highly impractical. To devise a temporally efficient model, we investigated the possibility of employing a 2-D model. Figure 2A shows that when RBCs are approximated by spheres and circles with identical SA/V, there is little difference in the predicted NO concentration profiles. Because SA/V equivalence allows the 2-D model to work as a suitable simplification of the 3-D model, it is unnecessary to model this system with the computationally intensive 3-D model.

In the interest of simplifying the model further, we developed a 1-D representation of a microvessel. In the 1-D model, the RBCs and plasma in the center of the vessel are approximated as a continuum, and the diffusional resistances to NO consumption by RBC-encapsulated HbO$_2$ are approximated by reducing the rate of reaction between NO and HbO$_2$.
(\(k_{NO+HbO_2}\)). In our study, we found that the 1-D model possessed two significant shortcomings. The first was that it was not possible to match the NO concentration profile from the 1-D simulation to the 2-D simulation in both the luminal and abluminal regions at the same time (Fig. 2C). The second was that for a constant \(k_{NO+HbO_2}\), the 1-D model deviated from the 2-D model as luminal radius increased (Fig. 2D). These shortcomings indicate that it is not possible to approximate the diffusional properties of NO in the particulate systems by reducing \(k_{NO+HbO_2}\) in the continuum system. On the other hand, the 2-D simplification from the 3-D model is satisfactory, as long as \(SA/V\) is used as the scaling factor.

**sGC activation.** Members of the NONOate class of NO donor have been shown to have potent vasodilatory properties (3, 17, 29, 30). To mediate sGC-dependent vasodilation, the NO donor must deliver enough NO to the smooth muscle to modulate sGC activity. Stone and Marletta (40) reported that the equilibrium dissociation constant of NO for activating sGC was \(\leq 250\) nM. A recent report by Condorelli and George (9) indicates that NO in the range of 5–100 nM influences the cGMP production rate of sGC. In this study, we selected a mean smooth muscle NO concentration (\(C_{NO,smc}\)) of 5 nM as the minimum concentration of NO required to modulate sGC.

In a microvessel with a 15-\(\mu\)m radius and a 1.5-\(\mu\)m glycocalyx, a steady-state NO production rate greater than 1 \(\mu\)M/s was required to raise \(C_{NO,smc}\) above 5 nM (Fig. 3A). An NO production rate of 1 \(\mu\)M/s is equivalent to a 1.7 nM plasma concentration of spermine NONOate (\(t_1/2 = 39\) min).

In an isolated rat femoral artery preconstricted with phenylephrine and NOS inhibited with \(N^o\)-nitro-L-arginine methyl ester, Miller et al. (29) found that spermine NONOate concentrations of at least 3 \(\mu\)M were required to induce vasodilation in the absence of RBCs or Hb. The large difference in our simulated results and the experimental results of Miller et al. (29) is partially due to the absence of RBCs in the assay. The presence of 10 \(\mu\)M HbO\(_2\) in the femoral artery drastically reduced the effectiveness of spermine NONOate. In addition, the relatively large lumen radius (\(r_{lu}\)) of the femoral artery (~350 \(\mu\)m) may have increased the effectiveness of the NONOate. In Fig. 3B, we show that for a constant NO production rate (1.54 \(\mu\)M/s), \(C_{NO,smc}\) increases with increasing \(r_{lu}\). This positive relationship between \(C_{NO,smc}\) and \(r_{lu}\) is due to the relationship between lumen volume and surface area. Because the lumen volume increases with the square of \(r_{lu}\), whereas the endothelial surface area increases in proportion to \(r_{lu}\), the strength of a volumetric source will vary more rapidly with \(r_{lu}\). In other words, the source increases faster than the relief, which results in a build up of NO.

**Impact of flow-induced RBC-free zone on NONOate efficacy.** As the size of the blood vessel approaches the size of the erythrocytes, the particulate nature of blood leads to the formation of a flow-induced RBC-free zone near the endothelium glycocalyx. Using experimental data from a number of publications, Sharan and Popel (38) developed a theoretical model to predict the RBC-free zone thickness as a function of Hct and vessel radius. When we increase the size of the cell-free zone for our reference case microvessel from 1.5 \(\mu\)m, used to approximate the glycocalyx, to 3.5 \(\mu\)m to approximate the glycocalyx and the flow-induced RBC-free zone, the concentration of NO in the abluminal region is almost doubled (Fig. 4A). This indicates the presence of flow in microvessels will enhance NONOate efficacy. However, as the size of the cell-free zone relative to \(r_{lu}\) decreases with increasing \(r_{lu}\) (38), NONOate efficacy may not be strongly influenced by flow in vessels with \(r_{lu}\) greater than a few hundred microns.

**Impact of extravasation on NONOate efficacy.** Submicron pores exist in the walls of a variety of microvessels (28). These pores facilitate the transport of small polar molecules from the vessel lumen into the interstitial space in the abluminal region. Because diffusion of the NONOate into the interstitial space will result in NO delivery adjacent to the smooth muscle, higher concentrations of NO will be delivered to the abluminal region.
tissue. Here we considered the case where the NONOate was uniformly dispersed in the luminal and interstitial plasma. Because there are a number of factors that will affect the distribution of unexpired donor, this scenario represents a maximum impact of extravasation on abluminal NO delivery. NONOates with short half-lives will probably expire before an equilibrium can be established between the lumen and interstitial space. If the rate of transport of the NONOate into the interstitial space is slow, relative to the NONOate release rate, then the concentrations of unexpired NONOate in the luminal and interstitial plasma will never be equal. Because the pore density varies across vascular beds, there is a significant possibility that the NONOates will be more effective in certain vascular beds (28).

Figure 5A indicates that extravasation of the NONOate into the interstitial space will increase abluminal NO concentrations by ~10% for a 15-μm radius microvessel with a 3.5-μm cell-free zone. Interestingly, the impact of extravasation on abluminal NO concentration increases with vessel radius (Fig. 5B). Extravasation augments the mean smooth muscle cell NO concentration by ~10%, for a 15-μm radius vessel, and ~20% for a 50-μm radius vessel. The increase in the effect of extravasation with increasing vessel radius is partially due to the increase in interstitial volume with radius. The strength of the NO source increases more rapidly than the surface area of the interstitial space.

In conclusion, here we have developed a model of NO-delivery by NONOates in resistance arterioles. During the model development, we found that it is reasonable to simplify a 3-D model of microvessels to a 2-D model. With this model, we identified three factors that will influence the ability of NONOates to deliver NO to abluminal regions. In particular, we found that abluminal delivery of NO increases with lumen radius, RBC-free zone thickness, and donor extravasation into the interstitial space. In Fig. 6, we illustrate the positive impact of the RBC-free zone thickness and NO donor extravasation on NO delivery to the abluminal region. Knowledge of the impact of these factors on NONOate efficacy should prove useful in the design of NONOates for research and clinical purposes.

GRANTS

This work was funded by National Institutes of Health Grant R01 HL-65741. DR Hyduke was supported in part by University of California, Los Angeles - National Science Foundation/Integrative Graduate Education and Research Training Program Award DGE-9987641.

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