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Endothelial NO and O₂^{•-} Production Rates Differentially Regulate Oxidative, Nitroxidative and Nitrosative Stress in the Microcirculation

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Abstract

Endothelial dysfunction causes an imbalance in endothelial NO and O₂^{•-} production rates and increased peroxynitrite formation. Peroxynitrite and its decomposition products cause multiple deleterious effects including tyrosine nitration of proteins, superoxide dismutase (SOD) inactivation and tissue damage. Studies have shown that peroxynitrite formation during endothelial dysfunction is strongly dependent on the NO and O₂^{•-} production rates. Previous experimental and modeling studies examining the role of NO and O₂^{•-} production imbalance on peroxynitrite formation showed different results in biological and synthetic systems. However, there is a lack of quantitative information about the formation and biological relevance of peroxynitrite in oxidative, nitroxidative and nitrosative stress conditions in the microcirculation. We developed a computational biotransport model to examine the role of endothelium NO and O₂^{•-} production on the complex biochemical NO and O₂^{•-} interactions in the microcirculation. We also modeled the effect of variability in SOD expression and activity during oxidative stress. The results showed that peroxynitrite concentration increased with increase in either O₂^{•-} to NO or NO to O₂^{•-} production rate ratios (Q_{O₂^{•-}}/Q_{NO} or Q_{NO}/Q_{O₂^{•-}}, respectively). The peroxynitrite concentrations were similar for both production rate ratios indicating that peroxynitrite related nitroxidative and nitrosative stresses may be similar in endothelial dysfunction or iNOS induced NO production. The endothelial peroxynitrite concentration increased with increase in both Q_{O₂^{•-}}/Q_{NO} or Q_{NO}/Q_{O₂^{•-}} ratios at SOD concentrations 0.1–100 μM. The absence of SOD may not mitigate the extent of peroxynitrite mediated toxicity as we predicted insignificant increase in peroxynitrite levels beyond Q_{O₂^{•-}}/Q_{NO} and Q_{NO}/Q_{O₂^{•-}} ratio of 1. The results support the experimental observations of biological systems and show that peroxynitrite formation increases with increase in either NO or O₂^{•-} production and excess NO production from iNOS or from NO donors during oxidative stress conditions does not reduce the extent of peroxynitrite mediated toxicity.

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Keywords

Peroxynitrite; NO; Superoxide; Production Rate; Biotransport; Superoxide Dismutase; Microcirculation; Flux; Computational Model; Endothelial Dysfunction

Introduction

Endothelial cells produce nitric oxide (NO) and basal levels of superoxide ($O_2^{\bullet-}$) under normal conditions. Both NO and $O_2^{\bullet-}$ act as essential signaling molecules [1, 2]. The key regulatory roles of NO in vasculature include vasodilation, vascular permeability regulation, prevention of leukocyte adhesion/platelet aggregation and prevention of smooth muscle cell proliferation [3]. $O_2^{\bullet-}$ directly or through its dismutation product H_2O_2 regulates vascular cell growth and angiogenesis [4, 5]. The regulatory roles of NO and $O_2^{\bullet-}$ are controlled by their production rates and their concentrations [6].

Disease conditions such as diabetes increase endothelial $O_2^{\bullet-}$ production and reduce NO availability [3, 7]. This condition is termed as endothelial dysfunction [3]. The increased $O_2^{\bullet-}$ production from uncoupled eNOS, mitochondria and NADPH oxidase [3, 7, 8] contributes to reduction in NO availability by reacting with NO to form peroxynitrite and by uncoupling eNOS [3, 9, 10]. Peroxynitrite through its decomposition products of nitrogen dioxide ($\bullet NO_2$), carbonate radical ($CO_3^{\bullet-}$) and hydroxyl radical ($\bullet OH$) causes multiple deleterious effects including tyrosine nitration of proteins [11], superoxide dismutase (SOD) inactivation [12, 13] and tissue damage [14]. Studies in synthetic experimental systems, *in silico* biological system and endothelial dysfunction have shown that peroxynitrite formation is strongly dependent on the NO and $O_2^{\bullet-}$ production rates [7, 15–20]. However, there is lack of quantitative information about the formation and biological relevance of peroxynitrite because of the difficult and indirect measurements.

In normal physiological conditions, NO is involved in vasodilation and prevention of leukocyte adhesion and activation [3, 21] whereas the $O_2^{\bullet-}$ levels are minimal due to the presence of SOD [18]. An imbalance in production of either NO or $O_2^{\bullet-}$ can initiate multiple pathological signaling events. These include an increase in cytokine and adhesion molecules expression from endothelial cells and the activation of protein kinase c (PKC) and mitogen activated protein kinase (MAPK) signaling pathways, PARP (poly (ADP-ribose) polymerase) enzyme and NF - κB transcription factor [5, 22–24]. Additionally, the enzyme inducible nitric oxide synthase (iNOS) expression and activity in endothelial cells increase [25, 26]. Increased iNOS expression in the endothelium can significantly increase endothelial NO and $O_2^{\bullet-}$ production [17, 27]. An increased NO and $O_2^{\bullet-}$ production results in severe oxidative, nitroxidative and nitrosative stress in the vasculature as observed in acute inflammatory state [24, 28].

In order to understand the effect of imbalance of NO and $O_2^{\bullet-}$, studies have used experimental synthetic systems [20, 29–31], kinetic models in synthetic systems [16, 17] and biotransport models in the microcirculation [32–35]. Experimental synthetic systems studies showed that the $O_2^{\bullet-}$ to NO or NO to $O_2^{\bullet-}$ production rate ratios ($Q_{O_2^{\bullet-}}/Q_{NO}$ or $Q_{NO}/Q_{O_2^{\bullet-}}$, respectively) of 1 yields maximum peroxynitrite concentration [20] or maximum tyrosine

nitration [29–31] and excess NO or $O_2^{\bullet-}$ production deactivates the tyrosine nitration process [29, 36] with the peroxynitrite concentration reaching a plateau beyond the production rate ratio of 1 [20]. This is contrary to the peroxynitrite mediated tyrosine nitration studies in biological systems where excess of either NO or $O_2^{\bullet-}$ production caused an increase in tyrosine nitration yields [37, 38].

To address this discrepancy, kinetic models [16, 39] simulated a more realistic system by incorporating SOD. They reported that an increase in the production rate ratios ($Q_{O_2^{\bullet-}}/Q_{NO}$ or $Q_{NO}/Q_{O_2^{\bullet-}}$) increases tyrosine nitration in the presence of SOD. However, in the absence of SOD, tyrosine nitration followed a bell shaped response with respect to production rate ratios. These studies suggested that the differences between synthetic and biological systems observation are attributed to (i) the formation of urate, which is a peroxynitrite scavenger [40, 41] produced by the $O_2^{\bullet-}$ generation system of xanthine oxidase/hypoxanthine [29, 42], (ii) a lack of nitration efficiency at low peroxynitrite concentration [30, 36, 43] and (iii) the presence of anti-oxidants including SOD and catalase and a role for NO diffusion [16]. It has been shown that the lack of a plateau of peroxynitrite (and thus bell shape in oxidation) with either SOD or rapid NO diffusion can in general be attributed to causing disappearance of NO/ $O_2^{\bullet-}$ by processes other than co-reaction [17]. It is therefore envisaged that the complex biochemical interactions between NO and $O_2^{\bullet-}$ are different in synthetic and biological systems.

Computational biotransport models of NO and $O_2^{\bullet-}$ interactions in isolated microvascular segments [32, 35, 44–48] studied several aspects. These include (i) varying the NO and $O_2^{\bullet-}$ production from the endothelium depending on the redox status of the endothelial cells [3, 32, 49], (ii) the reaction of NO with red blood cell (RBC) hemoglobin and consumption of NO by smooth muscle cells [45, 47, 50], and (iii) the role of SOD expression and activity, which can either decrease [51–53] or increase in vascular cells [54–57], extracellular regions [58] and in blood vessel segments [59, 60] under oxidative stress conditions. The biotransport models reported that (i) an increase in endothelial cell $O_2^{\bullet-}$ production significantly increased peroxynitrite concentration and reduced NO availability in the vasculature and (ii) increase in SOD levels significantly reduced peroxynitrite levels and increased NO availability in the vasculature. However, it is not known how the varying $Q_{O_2^{\bullet-}}/Q_{NO}$ or $Q_{NO}/Q_{O_2^{\bullet-}}$ ratios affects the peroxynitrite levels in the microcirculation.

To unravel the mechanisms responsible for the differences in the complex biochemical interactions between NO and $O_2^{\bullet-}$ in synthetic and biological systems, we developed a computational biotransport model to investigate the role of endothelium NO versus $O_2^{\bullet-}$ production rate ratio ($Q_{O_2^{\bullet-}}/Q_{NO}$ or $Q_{NO}/Q_{O_2^{\bullet-}}$) on the vascular NO, $O_2^{\bullet-}$ and peroxynitrite profiles at different regions of the vasculature. Previous biotransport models studied various aspects of the complex interactions at the microcirculation level including the vascular NO availability and increase in vascular oxidative, nitroxidative and nitrosative stress in scenarios such as leukocyte-endothelial cell interactions, leukocyte activation, SOD inactivation and tissue oxidative stress [32, 34, 45]. The $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio represent endothelial dysfunction conditions in which there is an increase in oxidative stress [3, 7] and an excess NO production from iNOS [61], respectively. Additionally, the microcirculation also involves multiple consumption routes of NO and $O_2^{\bullet-}$. We also

simulated the presence and absence of SOD. The NO, $O_2^{\bullet-}$ and peroxynitrite profiles can help identify the differences in the mechanisms involving NO and $O_2^{\bullet-}$ biochemical interactions in synthetic and biological systems.

Materials and Methods

Model Geometry

We developed a biotransport model for NO, $O_2^{\bullet-}$ and peroxynitrite in a blood vessel of 50 μm diameter and 500 μm length as shown in Figure 1. A concentric cylindrical geometry is used to represent the different regions of the blood vessel as shown in Figure 1. The regions include the red blood cell (RBC) rich region (CR) and the RBC free plasma region (CF) in the lumen, endothelium (E), interstitial space (IS), smooth muscle region (SM), non-perfused (NPT) and capillary perfused (PT) parenchymal tissue region.

To understand the complex biochemical interactions between these species (NO, $O_2^{\bullet-}$ and peroxynitrite), we simulated two cases. First, we kept endothelial NO production rate constant and varied $O_2^{\bullet-}$ production rate that is represented by endothelial $O_2^{\bullet-}$ to NO production rate ratio ($Q_{O_2^{\bullet-}}/Q_{NO}$). In the other case, we kept endothelial $O_2^{\bullet-}$ production rate constant and varied NO production rate that is represented by endothelial NO to $O_2^{\bullet-}$ production rate ratio ($Q_{NO}/Q_{O_2^{\bullet-}}$). The model solution predicts the concentration of NO, $O_2^{\bullet-}$ and peroxynitrite at any region of the vessel geometry. We evaluated the radial concentration profiles of NO, $O_2^{\bullet-}$ and peroxynitrite at the mid-point along the length of the vessel as shown in location P₁ of Figure 1. Obtaining concentration profile at location P₁ ensures that the blood flow profile is well developed and not affected by entrance effects. From the radial profiles, the maximum concentration of NO, $O_2^{\bullet-}$ and peroxynitrite at different geometrical regions of the vasculature (as described below) were obtained.

Governing equations

The governing equation for transport of NO, $O_2^{\bullet-}$ and peroxynitrite is a combination of diffusion, convection, generation and reaction kinetic terms. There were a total of three separate equations to account for the three species involved. The transport equation for NO, $O_2^{\bullet-}$ and peroxynitrite across different regions of the blood vessel is mathematically represented as:

$$\frac{1}{r} D_i \frac{\partial}{\partial r} \left(r \frac{\partial C_i}{\partial r} \right) - \sum R_{i,j} + \sum P_{i,j} = v_z \frac{\partial C_i}{\partial z} \quad (1)$$

The suffix i and j represents the different chemical species involved (NO, $O_2^{\bullet-}$ and peroxynitrite) and the different regions of the blood vessel (CR, CF, E, IS, NPT and PT), respectively. D_i ($\text{cm}^2.\text{s}^{-1}$) and C_i (nM) represents the diffusivity and concentration of species i, respectively. $R_{i,j}$ and $P_{i,j}$ ($\text{M}.\text{s}^{-1}$) represents the reaction rate and production of species i in region j, respectively. v_z ($\text{cm}.\text{s}^{-1}$) is the average blood velocity and is a function of maximum blood flow velocity ($v_{z\text{max}}$), radial distance r and radius of the blood vessel R (25 μm) [50, 62] as shown below:

$$v_z = v_{z\max} \left(1 - \frac{r^2}{R^2} \right) \quad (2)$$

The value of $v_{z\max}$ selected was 0.5 cm.s^{-1} on the basis of physiologically relevant value of wall shear stress due to blood flow (24 dyn.cm^{-2}), apparent blood viscosity (3 cPs) and vessel diameter ($50 \text{ }\mu\text{m}$) [50, 62, 63].

Boundary Conditions

Zero flux boundary conditions are applied at the vessel center and the outer surface of the parenchymal tissue (PT) region for all the species involved. Elsewhere, in the case of NO and $\text{O}_2^{\bullet-}$, the boundary condition for continuity of species flux and concentration is applied across all the interfaces between different regions of the blood vessel with the exception of the interface between cell free region (CF) and endothelium (E) and the interface between endothelium (E) and interstitial space (IS). The boundary condition between cell free region (CF) and endothelium (E) is represented as:

$$D_i \frac{\partial C_{i,\text{CF}}}{\partial r} - D_i \frac{\partial C_{i,\text{E}}}{\partial r} = P_{i,\text{E}} \quad (3)$$

The boundary condition between endothelium (E) and the interstitial space (IS) is represented as:

$$D_i \frac{\partial C_{i,\text{E}}}{\partial r} - D_i \frac{\partial C_{i,\text{IS}}}{\partial r} = P_{i,\text{E}} \quad (4)$$

The term $P_{i,\text{E}}$ in the above equations represents the endothelium based NO and $\text{O}_2^{\bullet-}$ production rates. In the case of peroxynitrite, the continuous boundary condition is applied across all the interfaces with the exception of the outer surface of PT region and vessel center, where zero flux boundary condition is applied.

Model Parameters

Parameters related to microvascular geometry are shown in Table 1. NO, $\text{O}_2^{\bullet-}$ and peroxynitrite undergo several reactions at all or specific regions in the blood vessel. The details of these reactions are shown in Table 2 and their associated kinetic parameters are shown in Table 1. The values of the rate constants shown in Table 1 are well established and have been used in previous computational models of NO, reactive oxygen species (ROS) and reactive nitrogen species (RNS) interactions in synthetic, cellular and microcirculation based systems with sufficient experimental validation [8, 64, 65] and sensitivity analysis data [19, 35]. The rationale behind use of some of these parameters can be found in earlier publications by Kavdia and co-workers [32, 46, 62]. The concentration of SOD across all the regions of the blood vessel was maintained at $1 \text{ }\mu\text{M}$ while analyzing the effect of $\text{QO}_2^{\bullet-}/\text{QNO}$ and $\text{QNO}/\text{QO}_2^{\bullet-}$ -ratio.

In order to analyze the effect of the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio, we selected a constant basal NO production rate in the E and PT regions for any $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio simulated. The basal NO production rate ($Q_{NO,j}$) used were 5.3×10^{-12} moles.cm⁻².s⁻¹ and 8.6×10^{-7} M.s⁻¹ for the E and PT regions, respectively based on literature sources [32, 46, 62, 66]. To analyze the effect of the $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio, we used constant basal $O_2^{\bullet-}$ production rate of 6.4×10^{-12} moles.cm⁻².s⁻¹ and 1.0×10^{-6} M.s⁻¹ for the E and PT regions, respectively. The $O_2^{\bullet-}$ production rate ($Q_{O_2^{\bullet-},j}$) for $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio and the NO production rate ($Q_{NO,j}$) for $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio in the E and PT regions were a factor of the basal NO and $O_2^{\bullet-}$ production rate, respectively and are shown in Table 3. Simulations were performed at $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ ratios of 0.05, 0.1, 0.2, 0.5, 0.75, 1.0, 1.2, 1.5, 2.0, 3.0 and 5.0, respectively.

$Q_{O_2^{\bullet-}}/Q_{NO}$ ratio beyond 0.2 represents endothelial oxidative stress conditions. Kar and Kavdia [8] showed that endothelial dysfunction is characterized by increased eNOS based $O_2^{\bullet-}$ production, which was about 27%–148385% of the eNOS based NO production. The $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio analysis represents advanced stages of endothelial dysfunction as basal $O_2^{\bullet-}$ production from the E and PT regions were approximately 116%–119% of the basal NO production used for the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio analysis. The NO production from the E and PT regions beyond the $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio of 1, were 116%–604% of the experimentally reported basal NO production rate [32, 46, 66]. Speyer et al. [27] reported a 200% increase in NO production and a significant increase in iNOS protein expression in mice endothelial cells subjected to inflammatory response. Thus NO production in E and PT regions beyond the $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio of 1, represents the iNOS related NO production.

SOD expression and activity are affected under oxidative, nitroxidative and nitrosative stress conditions [67–69]. Additionally, SOD was reported to reduce oxidative, nitroxidative and nitrosative stress in endothelial dysfunction [15, 19, 33, 70]. To account for the variation in SOD expression and activity and to examine the therapeutic effectiveness of SOD, we simulated the vessel NO, $O_2^{\bullet-}$ and peroxynitrite levels at SOD concentrations of 0.1, 1, 10 and 100 μ M. The analysis was performed at $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ ratios of 0.05, 0.75, 1.0, 2.0 and 5.0, respectively. The concentration of O_2 and CO_2 were selected based on previous biotransport models for O_2 and CO_2 in the microcirculation [71, 72].

Numerical Solution

The partial differential equations for transport of NO, $O_2^{\bullet-}$ and peroxynitrite with the appropriate boundary conditions were solved numerically using the finite element software Flex PDE 5.0 (PDE solutions Inc., Antioch, CA). The software uses adaptive meshing scheme, which generates finer mesh elements in regions of steep concentration gradient and coarse meshing elements elsewhere within the computational domain as shown in Figure 1. The relative accuracy used for all the simulations was set at 0.001.

Results

We obtained concentration profiles of NO, $O_2^{\bullet-}$ and peroxynitrite at specific regions of the vasculature (CR, CF, E, and SM regions). The maximum concentrations were from the radial concentration profiles of these species at location P_1 (Refer Figure 1). The concentrations in the Results section are the maximum concentrations unless stated

otherwise. We plotted the maximum concentrations as a function of the production rate ratio ($(Q_{O_2^{\bullet-}}/Q_{NO})$ or $(Q_{NO}/Q_{O_2^{\bullet-}})$) or as function of the production rate ratio and SOD concentration. We show concentration distribution and concentration profiles in Figures 2 and 3, respectively for $Q_{O_2^{\bullet-}}/Q_{NO}$ ratios of 0.05 and 5. An increase in the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio from 0.05 (Figures 2A–C and 3A) to 5 (Figures 2D–F and 3B) corresponds to a 100 fold increase in $O_2^{\bullet-}$ production with respect to unchanged rate of NO production. The results show that a 100 fold increase in $O_2^{\bullet-}$ production caused the NO concentration to decrease by approximately 80–90% whereas the $O_2^{\bullet-}$ and peroxynitrite concentration increased by 23–2500 fold and by 27–32 fold, respectively at all regions of the vasculature. Therefore increase in $O_2^{\bullet-}$ production corresponding to unchanged NO production rates can still significantly increase oxidative stress (through increased $O_2^{\bullet-}$ concentration), nitroxidative and nitrosative stress (through increased peroxynitrite concentration) in the vasculature.

Increase in $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio decreases NO concentration and increases $O_2^{\bullet-}$ and peroxynitrite concentration

Figure 4A, B and C show the concentration of NO, $O_2^{\bullet-}$ and peroxynitrite, respectively in the E, SM, CF and CR regions at location P_1 with respect to the change in the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio. The NO concentration decreased while the $O_2^{\bullet-}$ and peroxynitrite concentration increased with an increase in the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio at all the regions of interest (E, SM, CF and CR). An increase in the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio from 0.05 to 5, reduced the NO concentration by 80–83% at all the regions of interest. In addition, the NO concentration in the SM, E and CF regions were approximately 2 fold higher than the NO concentration in the CR region for any given $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio. This is due to the rapid consumption of NO by RBC based hemoglobin as observed in earlier studies [46, 50].

The $O_2^{\bullet-}$ concentration increased by 131, 148, 147 and 174 fold in the E, SM, CF and CR regions, respectively while the peroxynitrite concentration increased by 31 fold at all the regions of interest as the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio increased from 0.05 to 5. The CR region concentrations of $O_2^{\bullet-}$ and peroxynitrite were lower 3183–13167% and 36–38%, respectively than that of the respective E, SM and CF regions. This is due to extremely high reactivity of $O_2^{\bullet-}$, presence of SOD and consumption of NO by RBC based hemoglobin in the CR region. The lower % reduction in peroxynitrite concentration compared to the reduction in $O_2^{\bullet-}$ concentration shows that peroxynitrite can diffuse greater distances in the microvasculature compared to $O_2^{\bullet-}$.

Increase in $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio increases NO and peroxynitrite concentration and decreases $O_2^{\bullet-}$ concentration

For the $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio effect on profiles, we increased the NO production rate and maintained a constant rate of $O_2^{\bullet-}$ production (at oxidative stress level, $P_{O_2^{\bullet-},E}$ of 6.4×10^{-12} moles.cm⁻².s⁻¹). This analysis was performed to understand the impact of increased NO production under oxidative stress conditions. As seen in Figure 5, the NO concentration increased while the $O_2^{\bullet-}$ and peroxynitrite concentration decreased with an increase in the $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio at all the regions of interest. An increase in the $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio from 0.05 to 5 caused (i) the NO concentration to increase by 193, 215, 195 and 200 fold in the E, SM, CF and CR regions, respectively, (ii) the $O_2^{\bullet-}$ concentration to

reduce by 60%, 77%, 77% and 100% in the E, SM, CF and CR regions, respectively and (iii) the peroxynitrite concentration to increase by 33D36 fold at all the regions of interest. Additionally, the NO, $O_2^{\bullet-}$ and peroxynitrite concentration in the CR regions were 85–121%, 2648–361319% and 55–70% lower, respectively compared to the other regions of interest (E, SM and CF). These results show that the NO, $O_2^{\bullet-}$ and peroxynitrite concentration profiles are similar for all compartments except for CR. The possible explanation for low $O_2^{\bullet-}$ concentration in CR region are high reactivity of $O_2^{\bullet-}$, presence of SOD and increased NO availability in the CR and CF region at high $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio.

NO availability is independent of oxidative stress and $O_2^{\bullet-}$ concentration is independent of NO production at high SOD concentrations

To account for the variability in SOD expression and activity, we studied the effect of increased oxidative stress (indicated by an increase in the $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ ratios) and multiple SOD concentrations on the model predictions. In addition, peroxynitrite can nitrate tyrosine residues of SOD and therefore inactivate SOD. The reduced SOD concentration simulations can be used as an indicator of SOD inactivation and therefore as an indicator for these cases. We used lower SOD availability ($[SOD]=0.1 \mu M$), normal physiological SOD concentration ($[SOD]=1$ and $10 \mu M$) and increased expression or activity of SOD ($[SOD]=100 \mu M$).

Figure 6 shows the SOD dependent variation in the endothelial concentration of NO, $O_2^{\bullet-}$ and peroxynitrite with respect to the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio. For any given SOD concentration, the endothelial NO and $O_2^{\bullet-}$ concentrations decreased and increased, respectively, nonlinearly with an increase in the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio. The $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio change from 0.05 to 5 decreased the endothelial NO concentration 94%, 80%, 37% and 6% and increased the endothelial $O_2^{\bullet-}$ concentration 286, 130, 102 and 100 fold at SOD concentrations of 0.1, 1, 10 and 100 μM , respectively. Thus, increasing SOD greatly increases NO concentration (availability). SOD at high concentration of 100 μM does however eliminate the effects of oxidative stress (increasing $Q_{O_2^{\bullet-}}/Q_{NO}$) on NO availability.

On the other hand, an increase in the $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio increased the endothelial NO concentration and decreased the endothelial $O_2^{\bullet-}$ concentration for any SOD concentration as seen in Figure 7. The change in the $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio from 0.05 to 5 increased the endothelial NO concentration 627, 192, 106 and 100 fold and decreased the endothelial $O_2^{\bullet-}$ concentration 86%, 60%, 20% and 3% at SOD concentrations of 0.1, 1, 10 and 100 μM , respectively. SOD concentrations of 10 and 100 μM competes effectively with NO for $O_2^{\bullet-}$. Thus, $O_2^{\bullet-}$ concentration is relatively independent of increased NO concentration at SOD concentrations of 10 and 100 μM .

As seen in Figure 6 and Figure 7, the endothelial peroxynitrite concentration increased with increase in both $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ ratios at all SOD concentrations. Irrespective of the type of ratio, the fold increase in the endothelial peroxynitrite concentration was similar for a given SOD concentration. For the SOD concentrations of 0.1–100 μM , the endothelial peroxynitrite concentration increased 21, 30, 65 and 94 fold, and 27, 36, 68 and 94 fold as the $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio, respectively increased from 0.05 to 5.

In the absence of SOD, peroxynitrite concentration remains unchanged beyond $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio of 1 and changes insignificantly beyond $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio of 1

One of the focus of previous mathematical models was studying the effect of production rate ratios ($Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$) in the absence [17] and presence [16] of SOD. However, production rate ratio dependence on peroxynitrite concentration was only analyzed in the absence of SOD in kinetics models [17]. To understand how the $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratios affect the NO, $O_2^{\bullet-}$ and peroxynitrite concentration in the absence of SOD in a physiological biotransport model, we simulated SOD concentration of 0 μ M in all of the regions of the blood vessel.

Figure 8 and 9 show the concentration profiles in the E, SM, CF and CR regions with respect to $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio, respectively. In all of the regions of interest, the NO concentration decreased while the $O_2^{\bullet-}$ concentration increased with an increase in the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio. A 99% reduction in NO concentration and a 5035–94011 fold increase in $O_2^{\bullet-}$ concentration was observed in all the regions of interest as the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio increased from 0.05 to 5. An increase in the $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio increased NO concentration and decreased $O_2^{\bullet-}$ concentration. The increase in the $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio from 0.05 to 5 resulted in 4836–7733787 fold increase in the NO concentration and a 99% reduction in the $O_2^{\bullet-}$ concentration.

The peroxynitrite concentration increased by 16–19 fold in the E, SM, CF and CR regions as the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio increased from 0.05 to 1. However, beyond $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio of 1, the change in peroxynitrite concentration was insignificant. The maximum peroxynitrite concentration increased by 16–20 fold as the $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio increased from 0.05 to 1. In the CR region, the peroxynitrite concentration remained constant for $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratios greater than 1. In the CF, E and SM regions, the peroxynitrite concentration increased slightly as the $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio increased from 1 to 5 (~1.2 fold).

In the absence of SOD for the $Q_{O_2^{\bullet-}}/Q_{NO}$ or $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio of 1, the appearance of plateau in peroxynitrite concentration (see Figure 8C and 9C) is because of lack of $O_2^{\bullet-}$ consumption by processes other than NO reaction. Additionally, at any given production rate ratios ($Q_{O_2^{\bullet-}}/Q_{NO}$ or $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio), the peroxynitrite concentration in the CR region was 35%–73% lower compared to the peroxynitrite concentration in the CF, E and SM regions. This is due to the lower availability of NO in the CR region as shown in Figure 8A and Figure 9A.

Discussion

In this study, we investigated the role of endothelium based $O_2^{\bullet-}$ to NO or NO to $O_2^{\bullet-}$ production rate ratio on the NO, $O_2^{\bullet-}$ and peroxynitrite concentration profiles in a microvascular blood vessel. We also addressed the contradictory results observed between synthetic [16, 17, 29–31] and biological [37, 38] systems and identified the differences in the complex biochemical interactions between NO and $O_2^{\bullet-}$ for synthetic *in vitro* and biological *in vivo* scenarios.

An increase in peroxynitrite concentration is dependent on $O_2^{\bullet-}$ to NO or NO to $O_2^{\bullet-}$ production rate ratio

At any given SOD concentration, the peroxynitrite concentration in E, SM, CF and CR regions of the vasculature increased by 31 fold as the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio increased from 0.05 to 5. In these regions, the peroxynitrite concentration showed 33–36 fold increase for the same increase in the $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio. It has been reported that increase in $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio from 0 to 1 caused a reduction in SOD activity from 100% to 60% [39] while in another study a complete inactivation of SOD was reported on bolus addition of 0.5 mM peroxynitrite [13].

In the absence of SOD, the peroxynitrite concentration increased 16–19 and 16–20 fold as $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio, respectively were increased from 0.05 to 1; beyond the ratio of 1 peroxynitrite concentration did no change significantly. These results show that the increase in peroxynitrite concentration is similar irrespective of whether $O_2^{\bullet-}$ or NO is produced in excess ($Q_{O_2^{\bullet-}}/Q_{NO}$ or $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio). Our results agree with the existing complex computational modeling studies that include compartmentalization and diverse competitive scavenging [16, 17] only in the presence of SOD and NO diffusion. The increase in tyrosine nitration yields when the production rate of either NO or $O_2^{\bullet-}$ increase from the modeling study by Quijano et al. [16] are in agreement with our study. Our results are consistent with experimental observation of increased peroxynitrite concentration including peroxynitrite mediated nitrotyrosine formation in biological systems [37, 38, 73–75] and for synthetic experimental systems using simultaneous production sources of NO and $O_2^{\bullet-}$ in the absence of SOD [20]. Based on these data, we conclude that the peroxynitrite induced nitroxidative and nitrosative stresses will be similar and increase in endothelial dysfunction when either NO or $O_2^{\bullet-}$ are produced in excess. It should also be noted that the peroxynitrite concentration increased with increase in the production rate ratio $Q_{O_2^{\bullet-}}/Q_{NO}$ or vice versa in the absence of SOD. However, the increase in production rate ratio based peroxynitrite concentration ceases at equimolar production rates of NO and $O_2^{\bullet-}$.

NO and $O_2^{\bullet-}$ interactions in the microcirculation are different than that of in kinetic models

In addition to peroxynitrite, the variables of interest in the biotransport models and other kinetic models are the steady state concentration of NO and $O_2^{\bullet-}$. In this microcirculation model, the sources of NO and $O_2^{\bullet-}$ production are restricted to endothelium and the sinks of NO and $O_2^{\bullet-}$ are distributed throughout the system. Both NO and $O_2^{\bullet-}$ can be the limiting reactant for peroxynitrite formation depending on many factors.

The kinetic models developed for synthetic systems showed the varying dependence of the $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio on the steady state NO and $O_2^{\bullet-}$ concentration in the absence [16, 17] and presence [16] of SOD and NO diffusion. Quijano et al. [16] reported that only $O_2^{\bullet-}$ concentration increased linearly from 0 to 1.6 pM and NO concentration remained constant for the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio from 0 to 1.8 when NO diffusion and SOD were incorporated. In addition, they reported that only NO concentration increased linearly from 0 to 80 pM and $O_2^{\bullet-}$ concentration remained constant for the $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio from 0 to 1.8.

In absence of SOD and NO diffusion, Lancaster [17] and Quijano et al. [16] reported that increase in $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio from 0 to 1, reduced the NO concentration to zero which remained at this level for ratios beyond 1. On the other hand, the $O_2^{\bullet-}$ concentration remained 0 in the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio range of 0 to 1 and increased significantly beyond the ratio of 1. In addition, for the $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio range from 0 to 1, the $O_2^{\bullet-}$ concentration reduced to zero and the NO concentration remained zero. For $Q_{NO}/Q_{O_2^{\bullet-}}$ ratios beyond 1, the $O_2^{\bullet-}$ concentration remained zero and the NO concentration increased significantly. In the absence of SOD, experimental studies [30, 31, 76] reported a 3–16 fold increase in the rate of tyrosine nitration (indicator of peroxynitrite toxicity) and the modeling study by Lancaster [17] reported a 40 fold increase in peroxynitrite concentration as the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio increased from 0 to 1. The modeling predictions of peroxynitrite concentration in this study were similar in the absence of SOD (16–20 fold). In the absence of SOD, our model prediction showed that the NO and $O_2^{\bullet-}$ concentrations were greater than zero for the entire range of the $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio (0.05–5) in several regions of the microvessel, which is not in agreement with the previous modeling studies [16, 17].

Our modeling results are physiologically more relevant as we include NO consumption by RBC hemoglobin and smooth muscle cells. The competition from and compartmentalization of RBC hemoglobin [50, 77] and SOD for NO and $O_2^{\bullet-}$ consumption, respectively plays a role in the homeostasis of NO or $O_2^{\bullet-}$ in the vasculature. Under these circumstances, an increase in the $Q_{O_2^{\bullet-}}/Q_{NO}$ and $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio will increase the peroxynitrite concentration as shown in Figure 4C, 5C, 6C and 7C. Toledo Jr et al. [78] showed that peroxynitrite derived $\bullet NO_2$ radical related DAF2-DA (4–5-diaminofluorescein diacetate) nitrosylation increased initially with increasing NO concentration when sufficient SOD was present for $O_2^{\bullet-}$ scavenging. However, the presence of excess NO outcompeted SOD for consumption of $O_2^{\bullet-}$ and caused the product of DAF2-DA nitrosylation to reach a plateau phase. We used a constant O_2 concentration of 27 μM to react with NO in the vessels wall. NO consumption in the vessel wall and parenchymal cells may depend on O_2 concentration, which may vary across the wall [79, 80]. The reaction of peroxynitrite with glutathione (GSH) (present at mM levels) was neglected because of a two order magnitude slower reaction rate constant ($k=6.6 \times 10^2 M^{-1}.s^{-1}$) difference as compared to reaction rate constant with CO_2 ($k=1-2 \times 10^4 M^{-1}.s^{-1}$) [17, 81]. However, NO (indirectly through its oxidation product N_2O_3) and peroxynitrite (directly or through products of peroxynitrite decomposition and its reaction with CO_2) can nitrosate glutathione to form S-nitrosoglutathione (GSNO) [81–83], which is involved in a number of physiological processes including apoptosis, and response to oxidative and nitrosative stress [84, 85]. Overall, our results are in agreement with other studies that reported the lack of a plateau in peroxynitrite concentration [17]. The lack of a plateau in peroxynitrite concentration can be attributed to either SOD or rapid NO diffusion leading disappearance of NO/ $O_2^{\bullet-}$ by processes other than the reaction between NO/ $O_2^{\bullet-}$.

While products of peroxynitrite decomposition cause most of cellular injury, a quantitative indication of increase in peroxynitrite levels can give an indirect indication of cellular injury. Incorporating the products of reaction between peroxynitrite and CO_2 and peroxynitrite decomposition would entail solving six simultaneous transport equations corresponding to the six chemical species involved (NO, $O_2^{\bullet-}$, peroxynitrite, $\bullet NO_2$, $CO_3^{\bullet-}$ and $\bullet OH$). This would significantly increase the complexity of the problem and make it

computationally intensive as all of the governing equations will then be time and space dependent. In addition, the diffusion and reaction parameters of these species in different regions of the vessel will also be needed. This is beyond the scope of the current study.

SOD reduces vascular peroxynitrite levels while improving NO concentration and reducing $O_2^{\bullet-}$ concentration during endothelial oxidative stress conditions

In our predictions, an increase in oxidative stress (indicated by increase in the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio from 0.05 to 5) resulted in a 130–345 fold increase in $O_2^{\bullet-}$ levels, 30 fold increase in peroxynitrite levels and an 80%–83% reduction in NO levels of the vasculature. Additionally (see Figure 6), an increase in SOD concentration from 0.1 to 100 μM resulted in a 96% and 94% reduction in the endothelium based $O_2^{\bullet-}$ and peroxynitrite concentration and a 15 fold increase in endothelial NO concentration for $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio of 5. These results are similar to experiment observations and other modeling studies. Hink et al. [60] reported greater than 3 fold increase in $O_2^{\bullet-}$ levels and a 67% reduction in NO levels in diabetic mouse aortas compared to control conditions. Kar and Kavdia [45] reported a 4 fold increase in vascular $O_2^{\bullet-}$ and peroxynitrite levels and a 10% reduction in vascular NO levels on transition from normal physiology to endothelial oxidative stress conditions. Jackson et al. [86] showed that rabbit aortas subjected to a steady flux of $O_2^{\bullet-}$ ($2.3 \mu M \cdot min^{-1}$) had a significant decrease in acetylcholine dependent vasorelaxation (73% to 48% relaxation), which was almost restored by treatment with SOD (48% to 66% relaxation). Kar et al. [19] reported almost a 90% reduction in $O_2^{\bullet-}$ concentration corresponding to increase in SOD concentration from 0.1 to 100 μM during eNOS uncoupling. The above results show that the anti-oxidant SOD effectively also mitigates peroxynitrite production under endothelial oxidative stress conditions.

Endothelial dysfunction and its impact on vascular physiology

One of the consequences of endothelial dysfunction is eNOS uncoupling [3, 87]. Uncoupling of eNOS shifts the product of eNOS catalysis from nitric oxide to $O_2^{\bullet-}$ [7, 8, 88]. The extent of eNOS uncoupling is represented by the ratio of reduced form of bipterin to its oxidized forms [7, 8, 19, 88]. Additionally, endothelial dysfunction also induces endothelial $O_2^{\bullet-}$ production from other sources such as NADPH oxidase and mitochondria [3, 89]. In our study, the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio accounts for $O_2^{\bullet-}$ production from all the relevant endothelial sources during endothelial dysfunction. Our results show that increase in $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio from 0.05 to 5 significantly reduced the NO concentration and significantly increased the $O_2^{\bullet-}$ and peroxynitrite concentration in smooth muscle region, lumen and endothelium. Despite a significant reduction, the smooth muscle NO concentration (48 nM) is still well within the reported range of NO concentration required for vasodilation (23–100 nM) [46, 47]. However, increase in $O_2^{\bullet-}$ and peroxynitrite concentration in smooth muscle region can inhibit vasodilation [90, 91]. Increase in $O_2^{\bullet-}$ and peroxynitrite concentration in lumen primes and activate circulating leukocytes [45] while that in endothelium increases vascular permeability [92, 93]. Additionally, our results also showed that most increase in peroxynitrite concentration occurs when the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio increases from 0.05 to 2 (~22 fold). The increase in peroxynitrite concentration was insignificant beyond the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio of 2.

The physiological significance of the results in this study is summarized in Figure 10. The oxidative stress is simulated by an increase in the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio. In this study, the oxidative stress caused an increase in peroxynitrite concentration, and a reduction in NO availability. It has been reported that the oxidative stress induces increase in expression and activity of iNOS to compensate for a lack of vasodilation [94]. The iNOS induced increase in NO production (simulated as increase in the $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio) does not change peroxynitrite concentration as compared to that of the oxidative stress conditions in our study. An increase in peroxynitrite concentration results in a reduction in the SOD activity [13, 39]. In our study, the SM region NO concentration falls below the required NO concentration of 23 nM for vasodilation [47] at $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio of 1 in the absence of SOD. On the other hand, for $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio of 1 and beyond in the absence of SOD, the SM NO concentration reaches the vasodilation threshold concentration and continues to increase. However, SM peroxynitrite concentration for $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio of 1 and beyond in the absence of SOD is significantly higher compared to that of observed in the presence of SOD. Higher concentration in peroxynitrite in smooth muscle region contributes to vasoconstriction [90]. Thus, the absence of SOD may still lead to peroxynitrite mediated toxicity and may severely debilitate vasodilation.

Excess NO production from iNOS or from NO donors during oxidative stress conditions does not change the extent of peroxynitrite induced toxicity

An increase in vascular peroxynitrite levels can trigger increase in endothelial cell iNOS expression and activity leading to excess endothelial NO production during endothelial dysfunction [24]. Additionally, there were attempts to improve vascular NO availability during oxidative stress conditions by using NO donors [95, 96]. Both the above scenarios were addressed in our model by analyzing the effect of $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio on the vascular NO, $O_2^{\bullet-}$ and peroxynitrite levels. Our results showed that increasing both the $Q_{NO}/Q_{O_2^{\bullet-}}$ and $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio from 0.05 to 5 caused a similar increase in vascular peroxynitrite levels (33–36 fold). Therefore, despite an apparent increase in NO production during oxidative stress conditions (either by iNOS or from NO donors), the extent of peroxynitrite induced vascular damage is similar. Guzik et al. [95] reported almost a 3 fold increase in peroxynitrite formation and a 90% decrease in $O_2^{\bullet-}$ production in mammary arteries of patients undergoing coronary bypass surgery post addition of NO donor SNP (sodium nitroprusside). Mukhopadhyay et al. [96] reported a 2 fold increase in intracellular nitrotyrosine formation and cell apoptosis and a 4 fold increase in $O_2^{\bullet-}$ production in mice cardiomyocytes treated with 5 μ M doxorubicin. Addition of 100 μ M of NO donor (diethylenetriamine DETA NONOate) caused the nitrotyrosine formation and apoptosis to increase by 2 and 3 fold respectively. The $O_2^{\bullet-}$ production on the other hand reduced by 50%. No significant increase in nitrotyrosine formation and apoptosis was noticed upon addition of NO donors to untreated cells. Additionally, they also reported that use of iNOS inhibitors in doxorubicin treated cells caused a 67% reduction in apoptosis. The above studies in conjunction with our results from the $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio analysis indicates that excess NO production from iNOS or from NO donors during oxidative stress conditions may increase the extent of peroxynitrite induced toxicity initiated by endothelial dysfunction mediated oxidative stress and lower NO availability.

In conclusion, our results demonstrate that peroxynitrite formed from NO and $O_2^{\bullet-}$ interactions in an actual *in vivo* setup increases in concentration with increase in production rate of either NO or $O_2^{\bullet-}$. This is in contrast to synthetic *in vitro* systems where maximum peroxynitrite from NO and $O_2^{\bullet-}$ interaction is formed at equimolar production rates of NO and $O_2^{\bullet-}$. The differences occur due to more routes for NO and $O_2^{\bullet-}$ consumption such as reaction of NO with RBC hemoglobin, NO consumption by smooth muscle cells and $O_2^{\bullet-}$ consumption by SOD in *in vivo* settings compared to synthetic systems. Additionally, the results from the study show that increasing NO production/availability during endothelial dysfunction may cause the deleterious effects associated with increasing peroxynitrite concentration such as lipid/protein oxidation, peroxynitrite mediated protein tyrosine nitration and stress signaling pathway activation [11, 24, 96]. Therefore the complex interactions between NO and $O_2^{\bullet-}$ in the vasculature play a key role in redox sensitive vascular signal transduction. A more effective manner to reduce the deleterious effects of increasing peroxynitrite concentration during endothelial dysfunction would be to use NO donors in conjunction with an anti-oxidant such as SOD.

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Figure 1.

General schematic of the model geometry and the finite element meshing scheme (top right) with relative accuracy set to 0.001. The schematic shows the different regions of the blood vessel including luminal RBC rich core (CR) and RBC free plasma (CF) regions, endothelium (E), interstitial space (IS), smooth muscle region (SM), non-perfused parenchymal tissue region (NPT) and perfused parenchymal tissue (PT) region, respectively. P_1 represents the axial mid-point of the vessel and is the location where radial profiles of NO, $O_2^{\bullet-}$ and peroxynitrite were obtained.

Figure 2.

Concentration distribution of NO, $O_2^{\bullet-}$ and peroxynitrite at different regions of the microvasculature. **Panel A, B and C** shows the microvascular concentration distribution of NO, $O_2^{\bullet-}$ and peroxynitrite, respectively for the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio of 0.05. **Panel D, E and F** shows the microvascular concentration distribution of NO, $O_2^{\bullet-}$ and peroxynitrite, respectively for the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio of 5.

Figure 3. Radial concentration profiles of NO, $O_2^{\bullet-}$ and peroxynitrite at location P₁ for the $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio of 0.05 (**Panel A**) and 5 (**Panel B**), respectively.

Figure 4.

Effect of $Q_{O_2\bullet-}/Q_{NO}$ ratio on the concentration of NO, $O_2\bullet-$ and peroxynitrite in the E, SM, CF and CR regions of the blood vessel. **Panel A, B** and **C** shows the maximum radial concentration of NO, $O_2\bullet-$ and peroxynitrite, respectively at location P_1 in the E (blue line with solid diamonds), SM (brown line with solid squares), CF (purple line with solid circles) and CR (green line with solid triangles) regions with respect to $Q_{O_2\bullet-}/Q_{NO}$ ratio (individual values of $Q_{O_2\bullet-}/Q_{NO}$ ratio is represented by the individual solid diamonds, solid squares, solid circles and solid triangles).

Figure 5.

Effect of $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio on the concentration of NO, $O_2^{\bullet-}$ and peroxynitrite in the E, SM, CF and CR regions of the blood vessel. **Panel A, B and C** shows the maximum radial concentration of NO, $O_2^{\bullet-}$ and peroxynitrite, respectively at location P_1 in the E (blue line with solid diamonds), SM (brown line with solid squares), CF (purple line with solid circles) and CR (green line with solid triangles) regions with respect to $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio (individual values of $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio is represented by the individual solid diamonds, solid squares, solid circles and solid triangles).

Figure 6.

Effect of $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio and SOD on the concentration of NO, $O_2^{\bullet-}$ and peroxynitrite in the endothelium. **Panel A, B and C** shows the maximum NO, $O_2^{\bullet-}$ and peroxynitrite concentration, respectively in the endothelial (E) region with respect to $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio for SOD concentrations of 0.1 (blue line with solid diamonds), 1 (brown line with solid squares), 10 (green line with solid triangles) and 100 μ M (purple line with solid circles). Individual values of $Q_{O_2^{\bullet-}}/Q_{NO}$ ratio is represented by the individual solid diamonds, solid squares, solid circles and solid triangles.

Figure 7.

Effect of $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio and SOD on the concentration of NO, $O_2^{\bullet-}$ and peroxynitrite in the endothelium. **Panel A, B** and **C** shows the maximum NO, $O_2^{\bullet-}$ and peroxynitrite concentration, respectively in the endothelial (E) region with respect to $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio for SOD concentrations of 0.1 (blue line with solid diamonds), 1 (brown line with solid squares), 10 (green line with solid triangles) and 100 μM (purple line with solid circles). Individual values of $Q_{NO}/Q_{O_2^{\bullet-}}$ ratio is represented by the individual solid diamonds, solid squares, solid circles and solid triangles.

Figure 8.

Effect of $Q_{O_2\bullet^-}/Q_{NO}$ ratio on the concentration of NO, $O_2\bullet^-$ and peroxynitrite in the E, SM, CF and CR regions of the blood vessel in the absence of SOD. **Panel A, B** and **C** shows the maximum radial concentration of NO, $O_2\bullet^-$ and peroxynitrite, respectively at location P_1 in the E (blue line with solid diamonds), SM (brown line with solid squares), CF (purple line with solid circles) and CR (green line with solid triangles) regions with respect to $Q_{O_2\bullet^-}/Q_{NO}$ ratio (individual values of $Q_{O_2\bullet^-}/Q_{NO}$ ratio is represented by the individual solid diamonds, solid squares, solid circles and solid triangles).

Figure 9.

Effect of $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio on the concentration of NO, $O_2^{\bullet-}$ and peroxynitrite in the E, SM, CF and CR regions of the blood vessel in the absence of SOD. **Panel A, B** and **C** shows the maximum radial concentration of NO, $O_2^{\bullet-}$ and peroxynitrite, respectively at location P_1 in the E (blue line with solid diamonds), SM (brown line with solid squares), CF (purple line with solid circles) and CR (green line with solid triangles) regions with respect to $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio (individual values of $Q_{NO}/Q_{O_2^{\bullet-}}$ -ratio is represented by the individual solid diamonds, solid squares, solid circles and solid triangles).

Figure 10.

Schematic of key interactions involving NO, $O_2^{\bullet-}$, and peroxynitrite with associated rate constants. The red lines indicate inhibition in activity of enzymes (shown in light blue compartments), reduction in concentration of free radicals (orange compartments) and down-regulation of physiological response (light gray compartments). The green lines indicate an increase in concentration of free radical species (orange compartments), increase in expression and activity of enzymes (light blue compartments) and upregulation of physiological response (light gray compartments). The red compartment indicates various disease conditions resulting from upregulation/down-regulation of the various physiological responses shown (light gray compartments).

Table 1**Model Parameters**

Parameters	Values and Units	References
Systemic hematocrit	45%	[32, 50]
Capillary hematocrit	30%	[32, 46, 50]
C_{RBC-Hb}	20.3 mM	[32, 46, 50]
Blood vessel radius	25 μm	Text
CF region thickness	4.5 μm	[32, 50]
Endothelium thickness	0.5 μm	[32, 46, 50]
IS region thickness	0.5 μm	[32, 46, 50]
SM region thickness	6 μm	[32, 50]
NPT region thickness	30 μm	[32, 50]
O_2 concentration	27 μM	[32]
CO_2 concentration	1.1 mM	[32]
SOD concentration	0, 0.1, 1, 10 and 100 μM	[18, 32], Text
D_{NO}	$3.3 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$	[32, 46, 50]
$D_{O_2^{*-}}$	$2.8 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$	[32]
D_{Per}	$2.6 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$	[32]
f (tissue)	0.640	[32]
f (lumen)	0.817	[32]
v_{zmax}	0.5 $\text{cm} \cdot \text{s}^{-1}$	[50, 62], Text
k_{NO-RBC}	$0.2 \times 10^5 \text{ M}^{-1} \cdot \text{s}^{-1}$	[50]
k_{cap}	1.77 s^{-1}	[50]
k_{NO-O_2}	$9.6 \times 10^6 \text{ M}^{-2} \cdot \text{s}^{-1}$	[32, 50]
k_{SM}	$5 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}$	[32]
k_{Per}	$6.7 \times 10^9 \text{ M}^{-1} \cdot \text{s}^{-1}$	[32, 97]
k_{sod}	$1.6 \times 10^9 \text{ M}^{-1} \cdot \text{s}^{-1}$	[32, 97]
k_{oxy}	4.5 s^{-1}	[35]
k_{CO_2}	$5.6 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}$	[32]
k_{NO-Per}	$9.1 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}$	[32]
k_{self}	$2.54 \times 10^5 \text{ M}^{-1} \cdot \text{s}^{-1}$	[17]

Table 2

Description of reactions, kinetic expression and regional specificity used in the model

Reaction	Regions	Mathematical Expression	References
NO and Hb	CR	$-R_{NO,CR} = k_{NO-RBC} C_{RBC-Hb} C_{NO}$	[50]
NO and Hb	PT	$-R_{NO,PT} = k_{cap} C_{NO}$	[50]
NO and O ₂	CF, E, IS & NPT	$-R_{NO,j} = k_{NO-O_2} C_{NO}^2 C_{O_2}$	[32]
NO and sGC	SM	$-R_{NO,j} = k_{SM} C_{NO}^2$	[32]
NO and O ₂ ^{•-}	All	$-R_{NO,j} = R_{Per,j} = k_{Per} C_{NO} C_{O_2^{\bullet-}}$	[32]
O ₂ ^{•-} and SOD	All	$-R_{O_2^{\bullet-},j} = k_{sod} C_{O_2^{\bullet-}} C_{SOD}$	[32]
Peroxynitrite Decomposition	All	$f = \frac{1}{1 + 10^{pK_{Per} - pH}} = \frac{C_{ONOO^-}}{C_{ONOO^-} + C_{ONOOH}}$ $-R_{Per,j} = k_{Perd} (1 - f) C_{Per}$	[32]
Peroxynitrite and CO ₂	All	$-R_{Per,j} = k_{CO_2} f C_{Per} C_{CO_2}$	[32]
Peroxynitrite and NO	All	$-R_{NO,j} = -R_{Per,j} = k_{NO-Per} f C_{Per} C_{NO}$	[32]
O ₂ ^{•-} Self Dismutation	All	$-R_{O_2^{\bullet-},j} = k_{self} C_{O_2^{\bullet-}}^2$	[17, 97]

Table 3

NO and $O_2^{\bullet-}$ fluxes at the endothelium (E) and perfused parenchymal tissue (PT) region for analysis of production rate ratios

$Q_{O_2^{\bullet-}}/Q_{NO}$ or $Q_{NO}/Q_{O_2^{\bullet-}}$	$Q_{O_2^{\bullet-}}/Q_{NO}$		$Q_{NO}/Q_{O_2^{\bullet-}}$	
	$P_{NO,E} = 5.3 \times 10^{-12} \text{ moles.cm}^{-2}.\text{s}^{-1}$ $P_{NO,PT} = 8.6 \times 10^{-7} \text{ M.s}^{-1}$		$P_{O_2^{\bullet-},E} = 6.4 \times 10^{-12} \text{ moles.cm}^{-2}.\text{s}^{-1}$ $P_{O_2^{\bullet-},PT} = 1.0 \times 10^{-6} \text{ M.s}^{-1}$	
	$P_{O_2^{\bullet-},E} (\text{moles.cm}^{-2}.\text{s}^{-1})$	$P_{O_2^{\bullet-},PT} (\text{M.s}^{-1})$	$P_{NO,E} (\text{moles.cm}^{-2}.\text{s}^{-1})$	$P_{NO,PT} (\text{moles.cm}^{-2}.\text{s}^{-1})$
0.05	2.65×10^{-13}	4.3×10^{-8}	3.2×10^{-13}	5.0×10^{-8}
0.1	5.3×10^{-13}	9.0×10^{-8}	6.4×10^{-13}	1.0×10^{-7}
0.2	1.06×10^{-12}	2.0×10^{-7}	1.3×10^{-12}	2.0×10^{-7}
0.5	3.0×10^{-12}	4.3×10^{-7}	3.2×10^{-12}	5.0×10^{-7}
0.75	4.0×10^{-12}	6.4×10^{-7}	5.0×10^{-12}	8.0×10^{-7}
1.0	5.3×10^{-12}	8.6×10^{-7}	6.4×10^{-12}	1.0×10^{-6}
1.2	6.3×10^{-12}	1.0×10^{-6}	8.0×10^{-12}	1.2×10^{-6}
1.5	8×10^{-12}	1.3×10^{-6}	1.0×10^{-11}	1.5×10^{-6}
2.0	1.1×10^{-11}	2.0×10^{-6}	1.3×10^{-11}	2.0×10^{-6}
3.0	2.0×10^{-11}	3.0×10^{-6}	2.0×10^{-11}	3.0×10^{-6}
5.0	3.0×10^{-11}	4.3×10^{-6}	3.2×10^{-11}	5.0×10^{-6}