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COMPUTATIONAL MODELING OF OXIDATIVE STRESS: AN ANALYSIS OF NAD(P)H EFFECTS ON NITRIC OXIDE AND SUPEROXIDE DURING HYPERTENSION

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Abstract

Nitric oxide (NO) is inactivated in the human body when exposed to superoxide (O_2^-). This reaction forms peroxynitrite ($ONOO^-$). Superoxide is produced in the cardiac system by several mechanisms, including NAD(P)H oxidase. Superoxide dismutase (SOD) breaks down superoxide into oxygen and hydrogen peroxide. This prevents superoxide from reacting with nitric oxide and allows normal function to take place. Superoxide and peroxynitrite are main contributors to vascular disease in the human body, in particular hypertension. Experiments have shown that there is an increase of superoxide production in spontaneously hypertensive rats (SHR) vs. age-matched Wistar Kyoto rats (WKY) that were normotensive. The increase in superoxide production intensifies in the presence of scavenger DETCA Cu^{2+}/Zn^{2+} . A mathematical model has been developed by Kavdia and Popel to calculate concentrations of NO, $ONOO^-$, and O_2^- in the arterial and venule pair. Using this model we calculated the arterial and venule NO, $ONOO^-$, and O_2^- concentration profiles for normotension, hypertension, SOD inactivation, and NAD(P)H stimulated cases, and analyzed which specific regions showed amplification or reductions in concentrations. The inactivation of SOD allowed O_2^- concentration to significantly increase by 10-fold under basal conditions in hypertensive mice, while reducing the NO concentration in the model. Basilar arteries from hypertensive rats showed an increase of 4.1-fold in Nox4 compared to normotensive rats. The results suggest that the increase in superoxide in hypertensive rats is in correlation with the increase of NAD(P)H oxidase in these rats. The trends in superoxide production in this paper can help researchers and the medical community understand hypertension and vascular disease more thoroughly. Further, for future research, observed increases of Nox1 and Nox4 expression suggest specific regions where O_2^- will be high and which need to be evaluated.

Introduction

Cardiovascular disease is the leading cause of death in the United States. According to the American Heart Association, this disease was responsible for taking over 870,000 lives in 2005. Increased vascular production of reactive oxygen species (ROS) is a common characteristic of cardiovascular disease⁵. There are several factors that increase the risk of cardiovascular disease including hypertension, hypercholesterolemia, and diabetes mellitus. Work on the United States hypertension affects

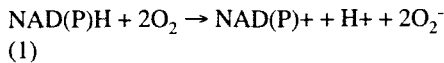
approximately 58 million Americans.

Vascular and cardiac tissues are rich sources of ROS, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and peroxynitrite ($ONOO^-$)⁷. Reactive oxygen species are the by-products of oxygen metabolism and are normally present in low levels of concentration inside the cells⁸. ROS are needed in aiding the signaling processes within the cells, and also in regulating vascular smooth muscle cell contraction and relaxation. Increasing amounts of O_2^- is the most common trend in vascular diseases, such as hypertension, because it causes oxidative stress in the vascular tissue⁹. One of the mechanisms for controlling oxidative stress in the vascular system is superoxide dismutase. Superoxide dismutase consumes O_2^- , and then converts it into less harmful compounds¹⁰.

The major sources of vascular superoxide include xanthine oxidase, nitric oxide synthases, mitochondrial oxidases, or NAD(P)H oxidases¹¹. Each of these sources generates superoxide in a different mannerism in the human body.

Xanthine oxidase (XO) is an iron sulfur flavoprotein that is found in high concentrations in the endothelial cells and plasma, but not in smooth muscle cells¹². Xanthine generates the superoxide by catalyzing hypoanthine and xanthine to form uric acid. Nitric oxide synthases (NOS), found predominantly in the endothelial cell region, play a major role in vascular diseases. Endothelial nitric oxidase synthases (eNOS) require tetrahydrobiopterin (BH_4) for the transfer of electrons to the nitrene of the L-arginine¹². This reaction under normal conditions forms nitric oxide in the vessels. However, when BH_4 is not present, eNOS generates O_2^- and H_2O_2 . Mitochondrial oxidases utilize the uncoupling of oxygen during mitochondrial oxidative phosphorylation, which occurs during the production of ATP, to synthesize O_2^- . The superoxide produced with lesion development in the arteries comes primarily from the mitochondrial oxidase¹².

In particular, in the work reported here, we analyzed more closely the NAD(P)H oxidase as a source of vascular superoxide. NAD(P)H oxidases are present in endothelial cells, fibroblasts, smooth muscle cells, neutrophils, and phagocytic mononuclear cells². The study of NAD(P)H started at the biochemical reaction of the compound in the cardiovascular system. NAD(P)H is a multi-subunit enzyme that catalyzes O_2^- production by reducing oxygen by one electron and using the NAD(P)H as the electron donor⁷.



One of the major sources of ROS formation is from the NAD(P)H oxidases. Endothelial cells and fibroblasts express both NAD(P)H oxidase 2 (Nox2) and NAD(P)H oxidase 4 (Nox4). Vascular smooth muscle cells express Nox1 and Nox4⁴.

In addition, nitric oxide released by endothelial cells is a key chemical that regulates blood flow. In oxidative stress conditions the availability of nitric oxides is reduced in vascular tissues, a process which is known as endothelial cell dysfunction¹⁰. With this dysfunction comes the natural response of the human body to overcome this production of superoxide. Superoxide is neutralized by a group of antioxidant enzymes. These enzymes include SOD, CAT, GPx, and thiol-disulfide oxidoreductases¹².

The research described here dealt primarily with SOD as the main antioxidant enzyme. There are at least three distinctive isoforms of SOD identified in humans: mitochondrial manganese-containing SOD (MnSOD, SOD2), the cytosolic copper/zinc-containing SOD (CuZnSOD, SOD1) and the extracellular SOD (eSOD, SOD3)¹³. SOD dismutates superoxide to form hydrogen peroxide and oxygen. Loss of endothelial NO available to endothelial cells is caused by the reaction with O_2^- . This reaction forms ONOO^- , which is a key component in many cardiovascular diseases such as hypertension, diabetes, and atherosclerosis. Understanding the levels of nitric oxide, superoxide, and peroxynitrite should contribute to a better understanding of the diseases and the oxidative stress state of the vascular system.

In 2004, Kavdia and Popel developed a mathematical model to create the concentration profiles for NO, ONOO^- , and O_2^- for an arterial and venule pair during microcirculation¹. This model defines the geometry of the arterial and venule vessels parallel to each other, while using diffusion rates and chemical reaction rates to calculate the concentration profiles.

The objective of this study was to predict NO, ONOO^- , and O_2^- concentration profiles for three different cases: 1) basal conditions compared to NAD(P)H stimulated conditions (2) basal conditions in normotensive and hypertensive rats with inactivation of SOD by DETCA (3) NAD(P)H stimulation in normotensive and hypertensive rats with inactivation of SOD by DETCA. We used data from Tamara Paravicini's "Increased NADPH-Oxidase Activity and Nox4 Expression during Chronic Hypertension is Associated with Enhanced Cerebral Vasodilatation to NADPH In Vivo". In their work, the arteries from Wistar-Kyoto rats (WKY) were compared to spontaneously hypertensive rats (SHR). The O_2^- production in these arteries was measured by 5 $\mu\text{mol/L}$ lucigenin-enhanced chemiluminescence under various conditions. The results from Paravicini's experiment are displayed in Figure 1. The trends in these data were applied to the Kavdia and Popel model for the purpose of the current research.

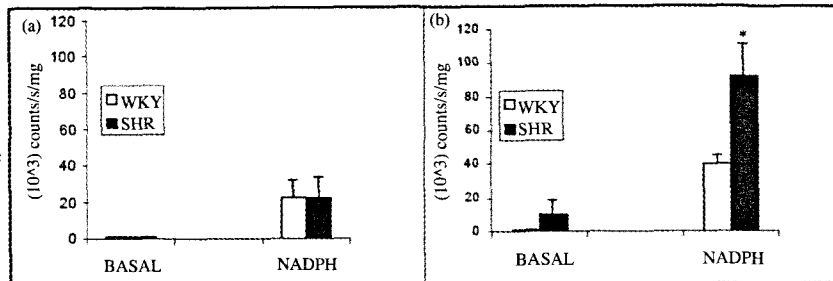


Figure 1. Data Results from Paravicini

Methods

Model Geometry

A previous model by Kavdia and Popel was used to simulate arteriole/venule during microcirculation. This model contains six different regions in the vessels: red blood cell rich (CR), red blood cell free (CF), endothelium (E), interstitial space (IS), smooth muscle (SM), and a nonperfused parenchymal tissue (NPT). The parenchymal tissue (PT) is the region around the arteriole and venule pair. Figure 2 shows the arterial and venule vessel next to each other. The regions have increasing diameters for each separate layer. Nitric Oxide (NO) is produced at the luminal and abluminal surfaces of the endothelium¹⁰.

The steady-state mass transport equation (cylindrical conditions) can be used to solve for the NO mass transport because the convective transport of NO can be neglected and the NO profiles reach steady state within milliseconds¹⁰.

$$D_j \nabla^2 C_j \pm \sum R_{j,i} = 0 \quad (2)$$

In this equation, j represents the particular model of interest; C_j is the concentration; D_j is the diffusivity; and $R_{j,i}$ stands for the production and consumption of the species due to chemical reactions.

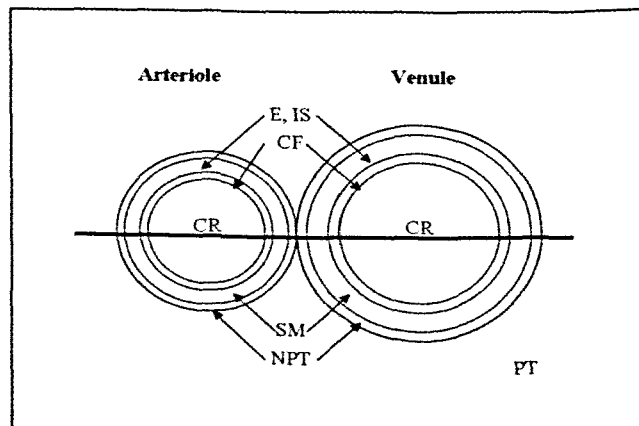


Figure 2: Arterial/Venule Geometry
Reference 10 (Kavdia M. A computational model for free radicals transport in the microcirculation. *Antioxid Redox Signal*. 2006;8(7-8):1103-1111.) 2

Boundary Conditions

Specific boundary conditions needed to be set to model this geometry and diffusion rates. At the outer edge of the PT, a zero-flux boundary condition was fixed, and at the interfaces with the endothelium, the release of NO and O₂⁻ were given by the following equations respectively¹⁰.

$$Q_j = D_j \frac{\partial C_{j,en}}{\partial r} - D_j \frac{\partial C_{j,is}}{\partial r} \tag{3}$$

$$Q_j = D_j \frac{\partial C_{j,cf}}{\partial r} - D_j \frac{\partial C_{j,en}}{\partial r} \tag{4}$$

The NO and O₂⁻ concentration profiles in the vascular tissue were obtained from these equations.

Chemical Reactions

The chemical reactions that are involved in the different layers of the arterial and venule are a mixture of first and second order reactions. Each region is discussed in further detail in Kavdia's "Venular endothelium-derived NO can affect paired arteriole: a computational model"¹⁴. The areas that are rich in red blood cells contain high levels of hemoglobin. This hemoglobin reacts at a high rate with the NO in the region, as seen in the following equation where k_{CR} is the effective NO reaction rate constant.

$$R_{NO,CR} = K_{CR}C_{NO} \tag{5}$$

In the CF region the chemical reactions are first order reactions because the hematocrit in this region is assumed to be zero¹⁰.

$$R_{NO,CR} = k_{CF}C_{NO} \tag{6}$$

In the remaining of the regions E, IS, and NPT the NO reaction is a second order reaction¹⁰.

$$R_{NO,I} = k_{O_2}C_{NO}^2C_{O_2} \tag{7}$$

Vascular smooth muscle sGC consumes the NO for the smooth muscle region (SM)¹⁰. Therefore the second-order reaction is:

$$R_{NO} = k_{SM}C_{NO}^2 \tag{8}$$

For the capillary-perfused PT region, the endothelial cells of the capillaries produce NO. For this region the reaction rate must take into account the amount of nitric oxide that is released by the capillary endothelial cell.

$$R_{NO} = k_{cap}C_{NO} - Q_{cap} \tag{9}$$

Each region is different, so each individual chemical reaction had to be considered when the model was derived.

Parameter Values

All parameters that were defined in the previous equations used in the model can be found in Table 1. The geometry of the arterial and venule vessels has been discussed in previous papers. The venule is assumed to be twice the size of the

arteriole. The arteriole is 25 μm and the venule is 50 μm in diameter. This ratio was assumed due to the reported distance between the arteriole and venule vessels¹⁵. The diffusivity rates of NO, O₂⁻, and peroxynitrite were assumed to be constant across the geometry and equal 3.3 x 10⁻⁵, 2.8 x 10⁻⁵, and 2.6x 10⁻⁵ cm²/s respectively according to Table 1. To determine the consumption of NO in CR region, Kavdia and Popel used a hematocrit of 0.45 in the region¹. The reaction rate for the consumption of NO is 1,270 s⁻¹ in the region⁹. The NO that is released by the capillary endothelial cell, k_{cap} was determined using a hematocrit of 0.3 and a capillary volume of 0.0146 cm³ for the model¹⁰. The k_{cap} calculated was 12.4 s⁻¹ for this case¹⁶.

Table 1. Model Parameters

Parameter	Value	Units	Reference
Systemic hematocrit	45	%	¹⁴
Capillary hematocrit	30	%	¹⁴
Arteriole radius	25	μm	¹⁴
RBC-free-layer thickness	4.5	μm	¹⁴
Endothelium thickness	0.5	μm	¹⁴
Interstitial Space thickness	0.5	μm	¹⁴
Smooth Muscle thickness	6	μm	²⁰
NPT thickness	30	μm	¹⁰
O ₂ concentration	27	μM	²¹
SOD concentration	1 (0.3)	μM	²²
CO ₂ concentration	1.14(0.114)	mM	
Half NO release rate, Q _{NO}	2.65 x 10 ⁻¹²	mol cm ⁻² s ⁻¹	²³
Half O ₂ ⁻ release rate, Q _{O₂⁻}	0.2 (2) x Q _{NO}	mol cm ⁻² s ⁻¹	¹⁴
D _{NO}	3.3 x 10 ⁻⁵	cm ² s ⁻¹	²⁴
D _{O₂⁻}	2.8 x 10 ⁻⁵	cm ² s ⁻¹	²⁵
D _{ONOO⁻}	2.6 x 10 ⁻⁵	cm ² s ⁻¹	²⁵
F(=C _{NO} /C _{NO}) in tissue	0.640		¹⁴
F(=C _{ONOO⁻} /C _{NO}) in tissue	0.817		¹⁴
Reaction rates of NO with			
O ₂ -k _{O₂} C _{NO} ² C _{O₂}	9.6 x 10 ⁶	M ³ s ⁻¹	²⁶
O ₂ ⁻ -k _{O₂⁻} C _{NO} C _{O₂⁻}	6.7 (16) x 10 ⁷	M ³ s ⁻¹	²⁷
sGC, -k _{sGC} C _{NO} ²	5 x 10 ⁴	M ³ s ⁻¹	²¹
RBC	1.4 x 10 ⁷	M ³ s ⁻¹	²⁸
RBC-rich core (CR) - k _{CR} C _{NO}	1,270	s ⁻¹	¹⁴
Capillaries, -k _{cap} C _{NO} +Q _{cap}	12.4 C _{NO} - 8.6 x 10 ⁻⁷	s ⁻¹	¹⁴
Reaction rates of O₂⁻ with			
SOD, -k _{SOD} C _{O₂⁻} C _{SOD}	1.6 x 10 ⁶	M ³ s ⁻¹	²⁹
Reaction rates of ONOO⁻ with			
CO ₂ -k _{CO₂} C _{ONOO⁻} C _{CO₂}	5.6 x 10 ⁴	M ³ s ⁻¹	³⁰
NO, -k _{NO} C _{NO} C _{NO}	9.1 x 10 ⁴	M ³ s ⁻¹	³¹

Numerical Solutions

Flex PDE 3.0 software was used for modeling the arteriolar and venular endothelial NO, O_2^- , and ONOO $^-$ concentrations. Flex PDE 3.0 is computer software that can be used for modeling and solving numerical problems.

Simulations

Tamara M. Paravicini's article "Increased NADPH-Oxidase Activity and Nox4 Expression during Chronic Hypertension is Associated with Enhanced Cerebral Vasodilatation to NADPH In Vivo" demonstrated experimental trends in the production of superoxide (O_2^-) in Wistar-Kyoto rats (WKY) and Spontaneously Hypertensive rats (SHR). This data was assumed for a model of the human arterial/venule microcirculation during normotension and hypertension. For the base case we used the whole tissue superoxide production as fraction $c = 0.2$ of NO production and used $10 \mu\text{M}$ of SOD.

The first simulation involved an increase of O_2^- production by 22-fold due to the NADPH stimulation of the arteriolar and venule. For this case the new whole tissue superoxide production fraction of NO production was $c = 4.4$. The SOD remained at $10 \mu\text{M}$. The results of both the normotensive and hypertensive were compared for basal and NADPH stimulated vessels.

The second simulation compared the three different basal conditions with a difference in the level of SOD. We used a condition with SOD of $10 \mu\text{M}$ and a superoxide production fraction $c = 0.2$ and compared it to the normotensive and hypertensive basal condition with an inactivation of SOD. The experiment used Cu^{2+} chelating agent diethyldithiocarbamic acid trihydrate (DETCA) to inactivate the $\text{Cu}^{2+}/\text{Zn}^{2+}$ SOD. We assumed that SOD was reduced and that the level changed from $10 \mu\text{M}$ to $1 \mu\text{M}$. In normotensive basal condition with reduction of SOD the superoxide production fraction remained at $c = 0.2$. For hypertensive basal conditions the superoxide production fraction was $c = 2$ due to a 10-fold increase in superoxide production.

The third simulation compared the NAD(P)H stimulated hypertensive/normotensive vessels with the change in SOD. With SOD of $10 \mu\text{M}$, the normotensive and hypertensive vessels both had a superoxide production fraction of $c = 4.4$, which are 22-fold greater than the basal conditions. With DETCA reduction of SOD to $1 \mu\text{M}$, the normotensive vessels had a superoxide production fraction of $c = 8$, which was a 40-fold increase from the basal conditions. With DETCA reduction of SOD to $1 \mu\text{M}$, the hypertensive vessels had a superoxide production fraction of $c = 18.4$, which was 2.3-fold greater than the normotensive NADPH stimulated conditions.

Results

Normotensive and Hypertensive Cases (Basal and NADPH excited) with SOD of $10 \mu\text{M}$

Profiles for the reactive oxidative species were generated according to the concentration values along the horizontal center axis of the geometry, as seen in Figure 2. The first

vertical line on the graph at 0.975 cm represented the middle of the arterial vessel. The endothelial cell region was $5 \mu\text{m}$, therefore the second line was hard to distinguish from the first because it was at 0.9775 cm on the graph. The third vertical line represented the smooth muscle cell region in the arterial vessel. The fourth vertical line represented the middle of the venule vessel at 0.105 cm on the graph.

For the base case, we used normal parameters as described in the methods section. The plots of NO, O_2^- , and ONOO $^-$ concentrations for the base case are displayed in Figures 3, 4 and 5, respectively. In Figure 3, the NO production of the Basal hypertensive and Basal normotensive with SOD of $10 \mu\text{M}$ is the same. This simulation of basal hypertensive and normotensive with SOD of $10 \mu\text{M}$ had the highest NO concentration and the lowest O_2^- concentration. The NAD(P)H stimulated hypertensive and normotensive with SOD of $10 \mu\text{M}$ were equal to each other. The NO concentration in the lumen of the CR region of both the arterial and venule was zero. As the NO profile approached the EN region, the concentration reached 100 nM. From that region, the NO concentration profile decreased back to 0 nM in the lumen of the venule vessel. The NAD(P)H stimulation during this case only caused a noticeable change in the NO profile in the PT region surrounding the arterial and venule pair.

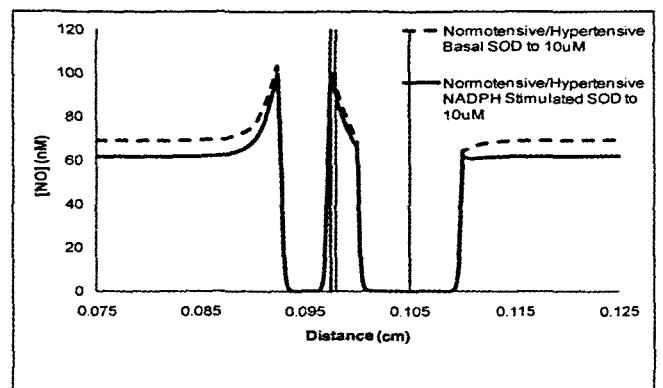


Figure 3. NO CONCENTRATION PROFILE FOR CASE 1

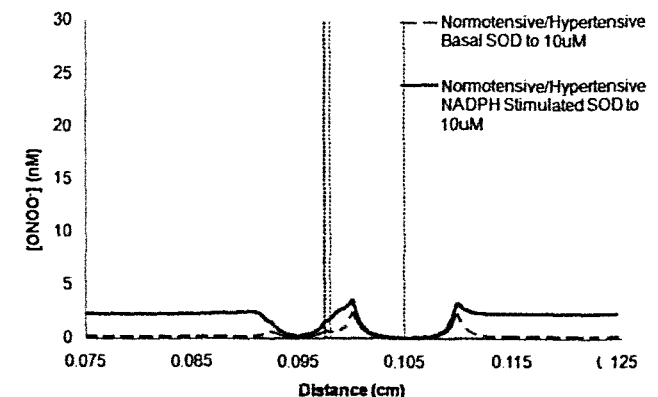


Figure 4. ONOO $^-$ CONCENTRATION PROFILE FOR CASE 1

The ONOO $^-$ concentration profile showed a visible change in Figure 4 throughout the arterial and venule pair due to

the NAD(P)H stimulation. This stimulation caused a 20-fold increase in the ONOO⁻ concentration. In the SM region, the ONOO⁻ concentration increased from 1 nM to 2.5 nM due to the NAD(P)H stimulation. The peroxynitrite concentration was 0 nM in the lumen of the venule vessel.

The O₂⁻ concentration profile is shown in Figure 5. This concentration profile shows that there was an increase in superoxide production in both the arterial and venule EN region. The O₂⁻ concentration had a peak at 0.433 nM and 2.29 nM in the arterial EN and venule EN, respectively.

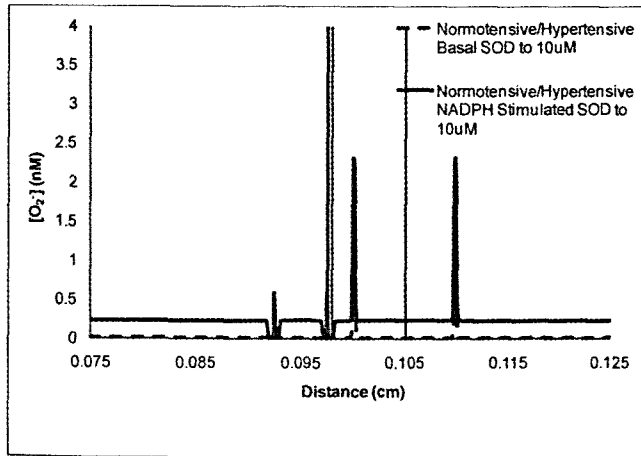
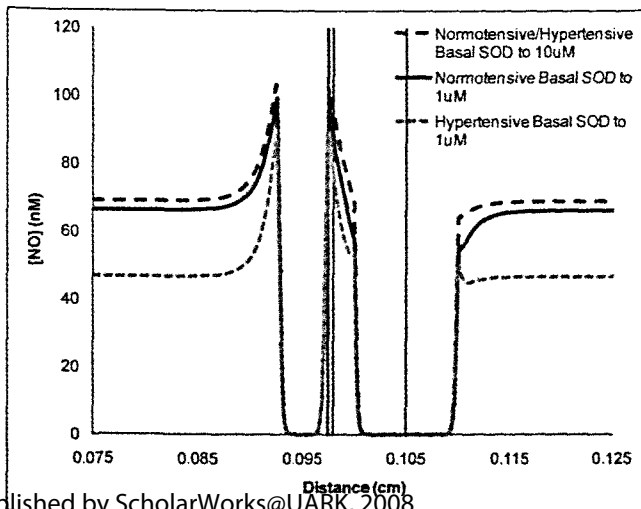


Figure 5. O₂⁻ CONCENTRATION PROFILE FOR CASE 1

Normotensive and Hypertensive Cases (Basal Only) with SOD being inactivated from 10 μM to 1 μM

We modeled the concentration profile of NO, ONOO⁻, and O₂⁻ for the basal case only of normotensive and hypertensive cases, but the SOD was reduced from 10 μM to 1 μM with DETCA Cu²⁺/Zn²⁺. The nitric oxide production in the reduced SOD was lower than that of the 10 μM SOD cases. Also the NO level for the hypertensive case with SOD of 1 μM was much lower than the normotensive case with SOD of 1 μM under basal conditions. Figure 6 shows that the highest



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Figure 6. NO CONCENTRATION PROFILE FOR CASE 2

concentrations of the NO were 102.3 nM, 96.1 nM, and 89.05 nM for Normotensive/Hypertensive basal case with SOD to 10 μM, Normotensive basal case SOD to 1 μM, and Hypertensive basal case with SOD to 1 μM, respectively in the EN arterial regions. The hypertensive basal case with SOD to 1 μM shows a NO concentration that was 20 nM less in the PT surrounding region of the arterial/venule pair compared to normotensive vessels in the same case.

The peroxynitrite concentration profile is shown in Figure 7. The hypertensive vessel with SOD reduction to 1 μM had the highest levels of ONOO⁻. This corresponds to the same case having the lowest levels of NO. The peak in the arterial vessel was in the EN and SM region. The concentration levels were 6.56 nM, 4.25 nM, and 0.647 nM in hypertensive basal case with SOD to 1 μM, normotensive basal case with SOD to 1 μM, and normotensive/hypertensive basal case with SOD to 10 μM, respectively. The peaks for the venule EN and SM region were 17.8 nM, 15.6 nM, and 2.36 nM, respectively.

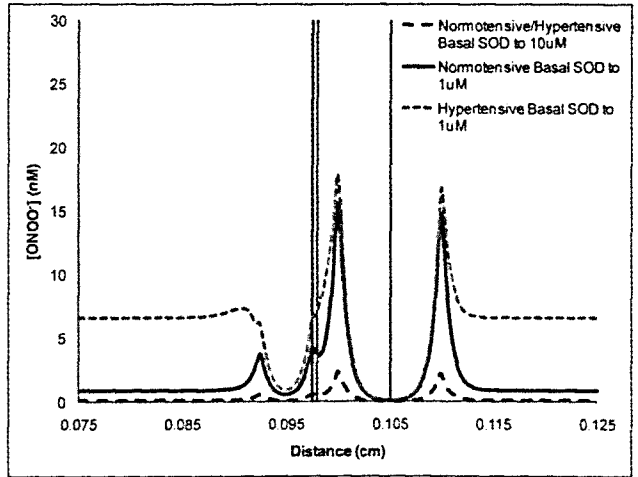


Figure 7. ONOO⁻ CONCENTRATION PROFILE FOR CASE 2

The superoxide concentration profiles for these specific cases are in Figure 8. This profile demonstrates that SOD reduction caused an increase of superoxide in the vessels by

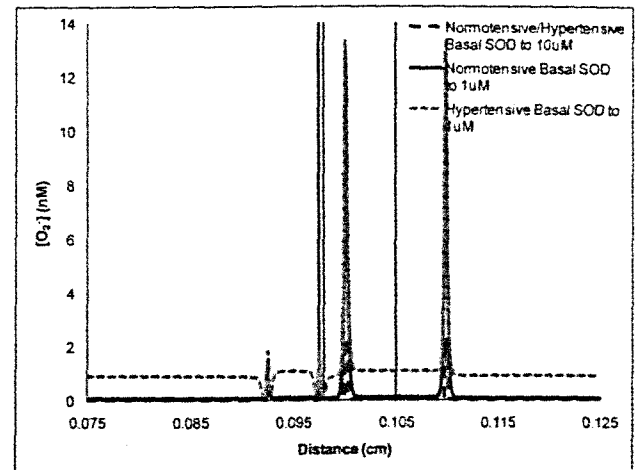


Figure 8. O₂⁻ CONCENTRATION PROFILE FOR CASE 2

10-fold in hypertensive vessels compared to the cases with SOD of $10\ \mu\text{M}$. In the hypertensive basal case with SOD reduced, the superoxide concentration was $1.81\ \text{nM}$ and $13.3\ \text{nM}$ in arterial and venule EN region, respectively. In comparison the arterial and venule EN region of normotensive basal with an SOD level of $1\ \mu\text{M}$ had peaks of $1.54\ \text{nM}$ and $12.83\ \text{nM}$, respectively.

Normotensive and Hypertensive Cases (NADPH excited) with SOD being inactivated from $10\ \mu\text{M}$ to $1\ \mu\text{M}$

Finally the profile for the NO, ONOO⁻, and O₂⁻ for normotensive and hypertensive vessels under NAD(P)H excited conditions with SOD reduced from $10\ \mu\text{M}$ to $1\ \mu\text{M}$. The hypertensive NAD(P)H excited vessels with an SOD of $1\ \mu\text{M}$ had the lowest concentration of nitric oxide. These results are shown in Figure 9. The endothelial region of the arterial vessel had the highest concentration of NO during microcirculation. During normotensive/hypertensive vessels with NAD(P)H stimulation and with SOD of $10\ \mu\text{M}$, the concentration was $99.6\ \text{nM}$. However, when SOD is reduced to $1\ \mu\text{M}$, the NO concentration in normotensive was $75.6\ \text{nM}$ and in hypertensive was $65.2\ \text{nM}$. The lumen of both the arterial/venule pairs was reduced to $0\ \text{nM}$ in all three cases.

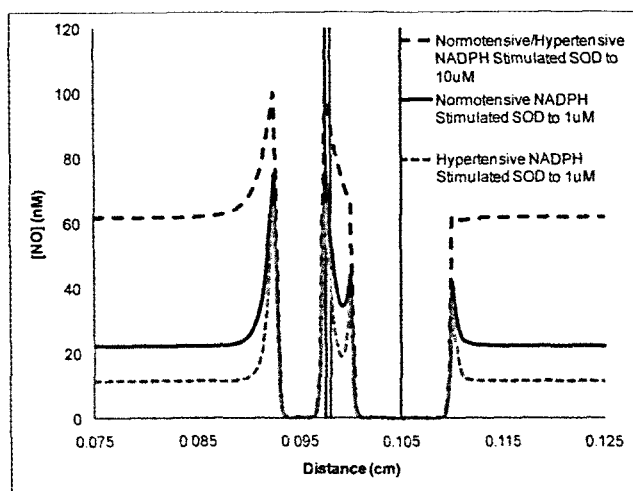


Figure 9. NO CONCENTRATION PROFILE FOR CASE 3

The ONOO⁻ concentration profile for the NAD(P)H cases with SOD reduction are presented in Figure 10. The reduced SOD, hypertensive vessels had the highest ONOO⁻ concentrations. The lowest concentrations of peroxynitrite were in the lumen of the venule. The highest concentrations of the ONOO⁻ were in the EN and SM region of the venule vessel. The concentrations were $29.4\ \text{nM}$, $23.3\ \text{nM}$, and $3.45\ \text{nM}$ for the reduced SOD hypertensive, reduced SOD normotensive, and the $10\ \mu\text{M}$ SOD normotensive/hypertensive, respectively.

The superoxide concentration profile for the NAD(P)H cases is shown in Figure 11. This profile shows that, when the SOD was reduced in the hypertensive vessels, the O₂⁻

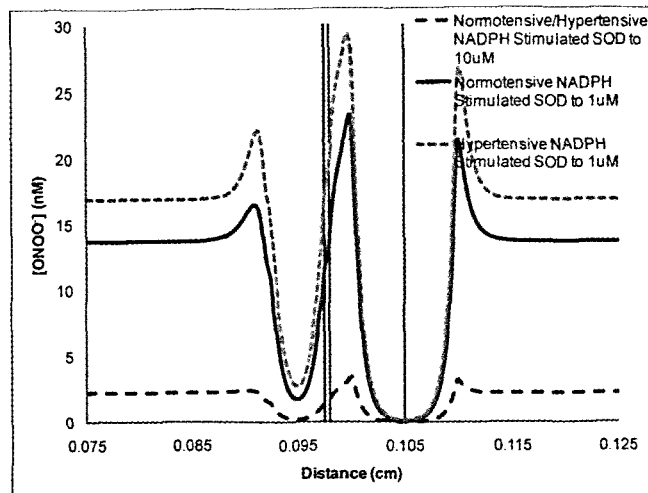


Figure 10. ONOO⁻ CONCENTRATION PROFILE FOR CASE 3

concentration peaks in the EN and SM of both the arterial and venule vessels. The arterial EN concentration level was lower than that of the venule because in the arterial the superoxide levels dropped dramatically before these regions. In hypertensive vessels with SOD reduction, the arterial EN superoxide concentration was $4.4\ \text{nM}$ and in the venule EN superoxide concentration was $17.5\ \text{nM}$. In normotensive vessels with SOD reduction, the arterial EN superoxide concentration was $2.9\ \text{nM}$ and in the venule EN superoxide concentration was $14.9\ \text{nM}$.

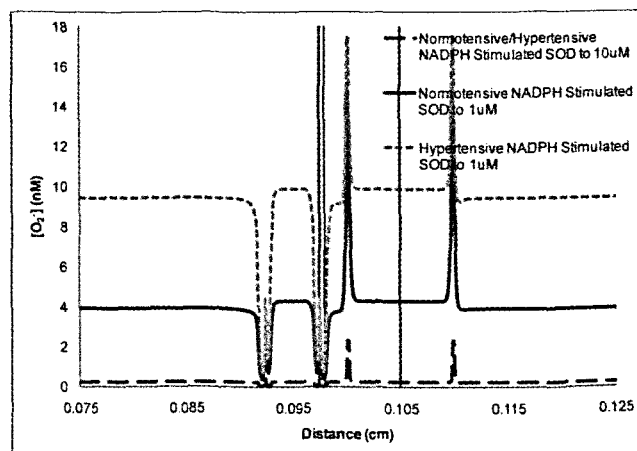


Figure 11. O₂⁻ CONCENTRATION PROFILE FOR CASE 3

Discussion

Effects of SOD Inactivation

SOD inactivation was shown to increase the superoxide production by 10-fold in Basal Hypertensive cases. This resulted in an increase in peroxynitrite but a decrease in the nitric oxide. SOD reduction with NAD(P)H stimulation caused an increase of 2.1-fold and 4.1-fold in superoxide production in normotensive and hypertensive vessels, respectively when compared to vessels with SOD of $10\ \mu\text{M}$. When SOD

was activated, the superoxide production was the same in hypertensive and normotensive vessels. This shows that SOD is very important in controlling O_2^- production and also in vascular disease. Without SOD, the level of superoxide may increase dramatically, potentially inducing vascular diseases. When SOD is $10 \mu\text{M}$, the vessels have a higher than normal level of H_2O_2 due to the dismutase of superoxide. This explains why the levels of H_2O_2 in plasma are higher than in normotensive patients³. This level of H_2O_2 , which is a powerful vasodilator, may have important consequences in the vascular system³. Current therapies for vascular disease, such as β -blockers, angiotensin antagonists, and angiotensin-converting enzyme inhibitors, act like antioxidants in some way¹⁷. New antioxidant therapies have the potential to be discovered to treat hypertension.

Effects of Normotension vs. Hypertension

Spontaneous hypertensive vessels can cause a 10-fold increase from normotensive vessels in basal conditions and a 2.3-fold increase from normotensive vessels in NAD(P)H stimulated vessels. Our data show that superoxide production is increased dramatically in hypertensive vessels. In hypertensive basilar vessels the Nox4 was 4.1-fold higher³. This shows that there is a direct correlation between the increase of NAD(P)H oxidase in regions and the increase of production with superoxide and vasodilation. In the area directly before the EN region, the superoxide level decreases dramatically. However, in the endothelial region, there is a large increase in superoxide production. This can be explained because this is where Nox4 is found to be most prevalent. Nox4 mRNA is seen to be 125-fold higher in endothelial cells than in smooth muscle cells² (see Table 2).

($\times 10^3/10^9$ copies 18S)	Nox4	Nox1
Monocyte	Undetectable	0.10
Endothelial Cells	270.0	0.87
Smooth Muscle Cells	2.15	0.22
Fibroblasts	6.25	0.45

Table 2. EXPRESSION OF NOX ISOFORMS

Also Nox4 and Nox1 mRNA levels are 2.5-fold and 10-fold greater in spontaneously hypertensive rats than in age-matched Wistar Kyoto rats, respectively¹⁸. Published data and the results from this model, showed a direct correlation between superoxide production and Nox expression.

Effects of NAD(P)H stimulation

When NAD(P)H was used to stimulate the vessels, it increased superoxide in the cells because the NAD(P)H reacted with the oxygen in the vessels. During this reaction, superoxide was produced, which significantly increased the levels of superoxide in the vessels. NAD(P)H stimulation increased the superoxide produce 22-fold compared to normal basal conditions when SOD was $10 \mu\text{M}$. During NAD(P)H

stimulation when SOD was reduced to $1 \mu\text{M}$, the superoxide production was 40-fold higher and 92-fold higher for normotensive and hypertensive, respectively when compared to normal basal conditions. In human coronary arteries, NAD(P)H stimulation is the major source of superoxide production, and in human coronary artery disease the NAD(P)H activity is significantly increased⁵.

Importance and Conclusion

The purpose of this model was to provide additional information about oxidative stress in hypertension. During hypertension, there was an increase in superoxide production when SOD was decreased. The trends from the NO, ONOO⁻, and O_2^- concentration profile show us which regions were affected by the disease and the physiological affects on the walls of the arteriole and venule. In addition, the H_2O_2 generated during superoxide dismutase may have a significant correlation to vasodilation during hypertension. The relevance of this research is underlined in the major role of oxidative stress in vascular diseases. The future of cardiovascular therapy is based on the balance between NO, ONOO⁻, and O_2^- within the body¹⁹.

Future Work

The data from this study could be used to further the understanding of vascular diseases. Nox1 and Nox4 are highest in the endothelial and smooth muscle cell region. The NAD(P)H oxidases are also expressed more in hypertension than during normotension. As previously stated, the expression of Nox1 is 10-fold greater and Nox4 is 2.5-fold greater. In recent years, there have been a number of publications about treating the arterioles and veins with different medications. Very similar tests have been done to measure superoxide production and Nox expression.

In one particular experiment, the vessels were treated with different hypertension medications: 1) high dose candesartan 2) low dose of candesartan 3) a dose of a combined hyralazine and hydrochlorothiazide¹⁸. All three treatments reduced the expression of Nox1 and Nox4. From the results of this modeling study, we can hypothesize that a reduction of Nox1 and Nox4 will also reduce the superoxide levels produced in the arterial and venule flow. The data show that the strong correlation might suggest a stronger link between NAD(P)H stimulation and the development of vascular diseases. In future research, we plan to model the concentration profiles of medically treated vessels to evaluate the effects of the medicine on the NO, ONOO⁻, and O_2^- profiles.

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Mentor Letter

Mahendra Kavdia speaks highly of Aaron Strobel's academic excellence and of the importance of the research described in this article.

Aaron started his research in my Laboratory in April, 2007. He obtained his undergraduate degree in Biological Engineering with a concentration in Biomedical Engineering/PreMed May, 08. He is one of the best students in our Department. Though the long-term plan of Aaron is to pursue a MD degree, he had an internship offer at St. Jude, San Francisco, CA but decided to work in bioinformatics area at Little Rock, AR to better prepare him for his long-term goals of pursuing MD. Aaron was motivated to perform research. He wrote an honors proposal and got funded in Spring, 08. He participated

in several research internships. The proposed research was to investigate the role of NADPH oxidase derived oxidative stress conditions on nitric oxide metabolism. This research is extremely important as oxidative stress is involved in numerous pathophysiological conditions such as cardiovascular diseases, diabetes, and aging. We had developed a computational model to understand the interaction of NADPH oxidase related oxidative stress on nitric oxide levels in vascular tissue. Aaron showed great initiative and also started communication with an experimental researcher in Australia to support his modeling research. His participation in my laboratory was very valuable for his basic knowledge of Biomedical Engineering and hands-on experience. Publication of this article in Inquiry certainly is a worthwhile recognition for Aaron's work and he deserves this.