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Mathematical and Computational Models of Oxidative and Nitrosative Stress

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Abstract

The importance of nitric oxide (NO), superoxide (O_2^-), and peroxynitrite ($ONOO^-$) interactions in physiologic functions and pathophysiological conditions such as cardiovascular disease, hypertension, and diabetes have been established extensively in in vivo and in vitro studies. Despite intense investigation of NO, O_2^- , and $ONOO^-$ biochemical interactions, fundamental questions regarding the role of these molecules remain unanswered. Mathematical models based on fundamental principles of mass balance and reaction kinetics have provided significant results in the case of NO. However, the models that include interaction of NO, O_2^- , and $ONOO^-$ have been few because of the complexity of these interactions. Not only do these mathematical and computational models provided quantitative knowledge of distributions and concentrations of NO, O_2^- , and $ONOO^-$ under normal physiologic and pathophysiologic conditions, they also can help to answer specific hypotheses. The focus of this review article is on the models that involve more than one of the 3 molecules (NO, O_2^- , and $ONOO^-$). Specifically, kinetic models of O_2^- dismutase and tyrosine nitration and biotransport models in the microcirculation are reviewed. In addition, integrated experimental and computational models of dynamics of NO/ O_2^- / $ONOO^-$ in diverse systems are reviewed.

Keywords

Nitric oxide; superoxide; peroxynitrite; hydrogen peroxide; superoxide dismutase; tyrosine nitration; endothelial cell; microcirculation; biotransport

I. INTRODUCTION

Nitric oxide (NO), reactive oxygen species (ROS), and reactive nitrogen species (RNS) are involved in numerous physiologic functions. NO, ROS, and RNS play a key role in signal transduction pathways, gene transcription, protein synthesis, and cell function. The interactions of NO, ROS, and RNS with biological systems lead to diverse outcomes that can be good or bad. An increasing body of evidence shows that ROS and RNS are important in the pathogenesis of many diseases including cardiovascular and neuronal diseases. Table 1 lists the literature reviews of NO, ROS, and RNS roles in various biological systems in the last decade. ROS and RNS are derived from partial reduction of oxygen and from NO,

respectively. Both ROS and RNS can be in free radical and non-free radical forms. From a physiologic and pathophysiologic perspective, important ROS are superoxide anion (O_2^-), hydroxyl radical ($OH^\bullet$), hydrogen peroxide (H_2O_2), and hypochlorous acid ($HOCl$), whereas important RNS are peroxynitrite ($ONOO^-$), nitrogen dioxide ($NO_2^\bullet$), and nitrous anhydride (N_2O_3).

II. NITRIC OXIDE, REACTIVE OXYGEN SPECIES, AND REACTIVE NITROGEN SPECIES

II.A. Nitric Oxide

NO, which is the endothelium-derived relaxing factor (EDRF), is a key signaling messenger in the cardiovascular systems for the regulation of blood pressure and vascular tone.¹⁻³ The discovery of NO as EDRF led to the awarding of the 1998 Nobel Prize in physiology or medicine to investigators F. Murad, R.F. Furchgott, and L.J. Ignarro. In addition, NO plays many important functions in cardiovascular systems including maintenance of vascular integrity⁴ and inhibition of platelet aggregation,⁵ leukocyte-endothelium adhesion,⁶ and vascular smooth muscle proliferation.⁷ A reduction in endothelial NO results in endothelial dysfunction, and abnormalities in NO signaling have been linked to many diseases including atherosclerosis, hypertension, hypercholesterolemia, diabetes mellitus, thrombosis, and stroke.⁸⁻¹⁶

NO is produced from the enzymatic oxidation of L-arginine to L-citrulline by several isoforms of NO synthase (NOS) enzymes,¹⁷ including neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). Recently, another NOS has been identified as mitochondrial NOS (mtNOS). These isoforms are divided based on their control of activity from intracellular calcium/calmodulin. The activity of nNOS and eNOS is dependent on a transient increase in intracellular calcium concentration, whereas the activity of iNOS is dependent at the level of transcription. NOS enzymes require several cofactors including tetrahydrobiopterin (BH_4), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), nicotinamide adenine dinucleotide phosphate (NAD(P)H), and molecular oxygen for their enzymatic activities.

From NOS isoforms, eNOS is the principal source of bioavailable NO in vasculature. NO produced by endothelial cells diffuses to vascular smooth muscle cells, where it activates soluble guanylate cyclase (sGC) that catalyzes the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), thus relaxing smooth muscle cells and causing vasodilatation. The majority of endothelial NO diffuses into the vascular lumen, where NO rapidly reacts with hemoglobin in red blood cells (RBCs) in flowing blood. The role of RBCs in NO metabolism has been a focus of many experimental investigations and several NO modeling papers (reviewed by Tsoukias¹⁸). Using an integrated approach of computational modeling and experiments, Deonikar and Kavdia¹⁹⁻²¹ have recently reported that (1) the effective reaction rate for NO and RBC interaction is $0.2 \times 10^5 \cdot M^{-1} \cdot s^{-1}$,¹⁹ (2) the RBC membrane permeability membrane between 0.0415 and 0.4 cm/s is required to maintain sufficient NO concentrations at the smooth muscle cell layer,²⁰ and (3) the downstream transport of NO is possible under the right circumstances.²¹ NO-sensitive dye

DAF-2 (4,5 diaminofluorescein) and DAF-FM and selective inhibitors of NOS has enabled understanding of NO-producing sites in living tissues.²²⁻²⁴ A role of nNOS-generated NO in vascular function has been demonstrated.^{25,26} The nNOS containing nerve fibers, nerve terminals, and mast cells have been reported as a source of vascular NO.²³ Non-neuronal cell types, including cardiac and skeletal myocytes, also have been shown to express nNOS. mtNOS, which is located in the inner mitochondrial membrane, modulates mitochondrial respiration and mitochondrial transmembrane potential. However, the contribution of mtNOS to tissue NO is controversial.²⁷ NO production related to iNOS can be up to 1000-fold higher than that of eNOS or nNOS. Macrophages, leukocytes, and fibroblasts express iNOS as a defense mechanism; however, prolonged activity leads to asthma, inflammation, and arthritis.

II.B. Reactive Oxygen Species

Under normal physiologic conditions, a small fraction of the inspired oxygen (O_2) used to generate energy by oxidative phosphorylation in mitochondria is converted to ROS. All cells of vascular tissue, including endothelial cells and smooth muscle cells, have enzyme systems that produce ROS. Primary ROS generated in the vascular tissue is O_2^- or H_2O_2 . Important ROS production sources include NAD(P)H oxidases, mitochondria, eNOS, and xanthine oxidase. Out of these sources of O_2^- production, the membrane-associated NAD(P)H oxidase is a major source of O_2^- in vascular diseases, including cardiovascular disease.^{28,29} Enzyme superoxide dismutase (SOD) catalyzes the conversion of O_2^- to H_2O_2 . H_2O_2 is converted to water by the enzymes catalase or glutathione peroxidase, which uses glutathione (GSH) as the reducing agent. O_2^- is highly reactive and has a very short half-life, whereas H_2O_2 is relatively stable and has a longer half-life.

In normal physiologic conditions, antioxidant systems protect against damage from ROS. These antioxidant systems include (1) antioxidants such as vitamins A, C, and E, and (2) enzyme systems such as SOD, glutathione peroxidase, and catalase. However, when ROS generation overwhelms the antioxidant defense, these radicals can alter cellular function by interacting with DNA, RNA, and fatty acids and can lead to apoptosis. This state is normally described as oxidative stress.³⁰ Both O_2^- and H_2O_2 can alter intracellular signaling pathways, including regulation of migration and host defense through oxidation. O_2^- can react with NO; thus, O_2^- and H_2O_2 levels can be controlled by NO, and oxidative damages are limited to specific cellular sites of ROS generation. However, O_2^- reaction with NO forms peroxynitrite (RNS), which destroys BH_4 ³¹ required for the NO synthesis. The depletion of BH_4 not only reduces NO synthesis but also results in uncoupling of eNOS that can lead to O_2^- production by eNOS.³²

II.C. Reactive Nitrogen Species

NO reacts rapidly with O_2^- to form strong oxidant peroxynitrite ($ONOO^-$).^{8,33} O_2^- reacts 3 to 6 times faster with NO ($k=9 \times 10^9 \cdot M^{-1} \cdot s^{-1}$) to form peroxynitrite than with SOD ($k=2 \times 10^9 \cdot M^{-1} \cdot s^{-1}$) to form H_2O_2 . Although NO concentration (order of few tens to hundreds of nanomolar³⁴⁻³⁷) in vivo is much lower than the SOD concentration (order of 10 millimolar³⁸), once formed, $ONOO^-$ contributes to vascular dysfunction indirectly by reducing NO bioavailability and initiating a number of pathological processes. In addition to

rapid reaction with O_2^- to form $ONOO^-$, NO also reacts with oxygen to form nitrous anhydride (N_2O_3) and nitrogen dioxide (NO_2). These RNS can initiate a number of pathological processes including lipid peroxidation, nitration of the protein tyrosine residues, inhibition of key metabolic enzymes, reduction of cellular antioxidant capacity by rapid oxidation of thiol, and DNA strand breaks leading to apoptosis.^{39,40} $ONOO^-$ also oxidizes BH_4 , an NOS cofactor, to dihydrobiopterin⁴¹, resulting in reduced NO formation.

II.D. Role of Mathematical Models in Oxidative and Nitrosative Stresses

Many aspects of NO, O_2^- , and $ONOO^-$ interactions in pathophysiologic conditions such as cardiovascular disease, hypertension, and diabetes have been studied extensively in many models both in vivo and in vitro in most circumstances. The literature is full of controversies that at times arise because of reductionist approaches in methods. These controversies include (1) the role of antioxidant vitamins A, C, and E^{42,43} in ameliorating oxidative stress and improving disease conditions, and (2) the potential of enzyme systems such as SOD^{44,45} and eNOS in causing or removing oxidative and nitrosative stresses in cardiovascular systems. The causes and consequences of oxidative and nitrosative stresses depend on a delicate balance among many processes. A better understanding of NO, ROS, and RNS in biological systems will require quantitative assessment of biochemical interactions and transport of these species from a systems perspective. Mathematical modeling approaches can help to elucidate behavior of these interactions for a particular system. Though experimental studies involving the 3 important molecules (NO, O_2^- , and $ONOO^-$) are many, the mathematical models have been limited because of complexities of biochemical interactions. In this article, mathematical models that involve more than one of the 3 molecules (NO, O_2^- , and $ONOO^-$) are reviewed.

III. Kinetic Models

One of the common themes of the kinetic models is to understand how the fluxes of NO and O_2^- affect a particular outcome. The kinetic models of NO, O_2^- , and $ONOO^-$ studied diverse aspects of roles of these molecules, which included (1) the kinetics of reaction of NO and superoxide,⁴⁶⁻⁴⁸ (2) superoxide dismutation catalyzed by superoxide dismutase,^{44,45,49,50} (3) role of GSH,⁴⁶ and (4) models of O_2^- production from mitochondrial transport chain.

III.A. Role of Superoxide Dismutase

Three types of SODs have been identified: cytosolic CuZn-SOD,^{51,52} mitochondrial Mn-SOD, and extracellular SOD. All SODs catalyze the dismutation of O_2^- according to Reaction 1 and play an important role in modulating oxidative stress and nitrosative stress levels.

The product of this reaction is H_2O_2 . H_2O_2 is converted to O_2 and H_2O by enzyme catalase. SOD can play an important role in increasing NO levels by reducing.

Approximately 80% of all SOD activity can be attributed to CuZn-SOD,⁵³⁻⁵⁵ and its deficiency leads to impaired NO-mediated arterial relaxation.⁵³ Mn-SOD protects against the mitochondrial electron transport chain O_2^- production.^{33,56} Blood vessels contain smaller amounts of Mn-SOD compared with CuZn-SOD and extracellular SOD; however, endothelium may contain high levels of Mn-SOD.^{53,55,57} Mn-SOD differs from the other 2 isoforms in that Mn-SOD-knockout mice die less than 3 weeks after birth.^{58,59} Vascular smooth muscle cells produce extracellular SOD, which is found in high amounts in the interstitial space.^{53,55,60,61} Extracellular SOD plays an important role in the regulation of O_2^- .^{62,63}

An overproduction of SOD or administration of high doses of SOD is reported to cause deleterious consequences, including cancer cell growth and ischemia reperfusion injuries, in a number of experimental studies.^{45,64,65} The possible explanation for SOD-induced toxicity is an increased production of H_2O_2 in the presence of higher SOD. Most mathematical models involving SOD^{44,45,49,50,66,67} attempted to provide an explanation of the experimental observation of increased cell and tissue damage upon overexpression and administration of high levels of SOD (reviewed by McCord⁴⁵). Results from mathematical models mostly suggested that increased levels of SOD in most cases decrease the production of H_2O_2 .^{44,50,67} The decrease in the production of H_2O_2 is attributed to a reduction in steady state O_2^- concentration.

Using simple models, Gardner et al.⁶⁷ and Licochev and Fridovich⁶⁸ considered the effects of varying SOD from zero to normal concentrations but not pathophysiologic concentration of SOD on H_2O_2 production. They concluded that SOD activity affects H_2O_2 production only when most O_2^- is eliminated through H_2O_2 free processes. To provide an explanation for increased oxidative stress, Kowald et al.⁵⁰ considered SOD to act as peroxidase and O_2^- reductase. In addition, they considered SOD's role to increase lipid peroxidation by removing O_2^- , which serves as a terminator for lipid peroxidation. For peroxidase function, SOD reacts with H_2O_2 to become a free radical generator. For O_2^- reductase function, SOD produces one H_2O_2 instead of the normal 2 H_2O_2 for every O_2^- reacted, which is termed *SOD cycle short circuiting*. In addition, they considered the O_2^- radical to have alternative reaction pathways. All reactions of O_2^- in vivo except with SOD were lumped in these alternative pathways. They concluded that the alternative pathway mechanism and SOD as O_2^- reductase is the most viable explanation for SOD-associated oxidative stress.

Buettener et al.⁶⁶, like others, also showed that the SOD can increase the flux of H_2O_2 . To understand how SOD can increase the production of H_2O_2 , they developed a kinetic model of O_2^- -peroxide system containing 16 nonlinear differential equations from the generation of O_2^- radical to the final conversion of H_2O_2 to water inside mitochondria. The main parameters for the model were equilibrium-specific formation of superoxide (K), and SOD concentration. The equilibrium-specific formation was varied between 0.001 and 1000 by varying the reverse rate constant, and SOD concentration was varied between 0.7 and 10 μ M. The model predicted that the SOD increases the steady state H_2O_2 level for $K < 1$ and has no effect on H_2O_2 level for $K > 1$. The $K < 1$ and $K > 1$ are identified as O_2^- -producing sources of mitochondrial electron transport chain and xanthine oxidase, respectively. However, Licochev and Fridovich⁴⁴ reported that the increased H_2O_2 formation observed by

Buettner et al.⁶⁶ is a result of the inclusion of dismutation reaction and not caused by an SOD-dependent increase in O_2^- formation.

III.B. Models of Tyrosine Nitration

Free or protein tyrosine residues nitration is a covalent posttranslational modification from nitrating agents that is observed in many disease conditions.⁶⁹⁻⁷² The nitration pathways involving $ONOO^-$ results in the formation of oxometal complexes and $ONOOCO_2^-$ that decomposes to carbonate radical (CO_3^-) and NO_2 . NO_2 oxidizes tyrosine to tyrosyl radicals and reacts with tyrosyl radicals to form 3-nitrotyrosine.⁷³ Because of the diffusion-limited reaction of $NO\cdot$ with O_2^- and the ability of $ONOO^-$ reaction product to tyrosine nitration in vitro, the formation of 3-nitrotyrosine is commonly used as an indirect marker for in vivo local formation of $ONOO^-$.

The focus of the tyrosine nitration computational models has been on whether $ONOO^-$ can perform as a biological nitration mediator.^{47,74} This question is raised because of apparent conflicting observations in nitration yield in homogenous and biological systems. Alternative pathways for tyrosine nitration also have been proposed, including transition metal-dependent mechanisms and heme-peroxidases such as myeloperoxidase and eosinophil peroxidase catalyzing free and protein tyrosine in the presence of nitrite and H_2O_2 . In homogeneous systems, the maximal nitration yields are achieved at the $NO:O_2^-$ flux ratio of approximately one,^{75,76} and an excess of either NO or O_2^- results in a decrease in nitration yields. A possible explanation for the reduced yield with an excess of NO or O_2^- may be deactivation of nitration with excess NO and O_2^- interactions with NO_2 and tyrosyl radicals, respectively. However, the nitration in biological systems increases with increase in either NO or O_2^- .^{77,78} This contradiction invalidates the use of 3-nitrotyrosine as a marker for $ONOO^-$.

To resolve the apparent conflict in nitration yield between the in vivo and in vitro data, Quijano et al.⁷⁴ modeled the 3-nitrotyrosine formation using simultaneous fluxes of NO and O_2^- . They hypothesized that the presence of SOD and the transmembrane diffusion of NO prevents the accumulation of NO and O_2^- and, therefore, the formation of $ONOO^-$. The transmembrane NO diffusion was represented with a rapid NO disappearance rate of 400 s^{-1} . The inclusion of transmembrane NO diffusion and SOD reduces NO concentration from $12\text{ }\mu\text{M}$ to 40 pM and O_2^- concentration from 200 nM to 0.8 pM , respectively. Their data showed that in biological systems $ONOO^-$ -mediated nitration is a low-yield process but responsive to increases in formation of NO and O_2^- . Both the incorporation of SOD and diffusion of NO increased tyrosine nitration formation when the ratio of fluxes of NO and O_2^- increased beyond one. However, the nitration yields were significantly lower in the range of femtomolar per minute when both SOD and diffusion of NO were incorporated compared with nanomolar per minute range when either SOD or diffusion of NO was incorporated.

Lancaster⁴⁷ systematically investigated oxidation, nitrosation, and nitration pathways to better understand the dominant reaction pathways and intermediate and product formations. The analysis indicated that oxidation, compared with nitrosation and nitration, is the dominant pathway for the fate of NO and O_2^- . When either flux of NO or O_2^- varies and the

other flux is constant, there is a linear increase in ONOO^- concentration that reaches a plateau in certain conditions. However, the ONOO^- concentration does not decrease with varying NO and O_2^- flux as required for the bellshaped curve.

It is still debatable whether ONOO^- is the exclusive source of tyrosine nitration in vivo. The alternative reaction mechanisms, which are normally ignored, need to be considered in tyrosine nitration in vivo, including a role for NO_2^+ . The nitration modeling has been restricted to only tyrosine nitration. It also can be extended to nitration of tryptophan residues, DNA bases, and sugars.

IV. BIOTRANSPORT/DIFFUSION MODELS OF NITRIC OXIDE, REACTIVE OXYGEN SPECIES, AND REACTIVE NITROGEN SPECIES IN THE MICROCIRCULATION

A common event in pathophysiologic conditions such as cardiovascular disease, hypertension, and diabetes is the reduction in bioactive NO availability, which is known as the endothelial dysfunction or impaired vasodilation.⁷⁹ Many hypotheses for the cause of the endothelial dysfunction have been proposed, including lower NO formation, higher ROS formation, higher interactions among NO and ROS, alterations in cellular signaling, and the inability of endogenous antioxidant. A role of reduced availability of NOS substrates and cofactors such as oxygen, tetrahydrobiopterin, and L-arginine also has been established. Though the role of NO , O_2^- and ONOO^- interactions have been studied extensively in vivo and in vitro in normal and pathophysiologic conditions; however, the quantitative information of these interactions in the microcirculation is not available. Over last decade, I and others have developed computational and mathematical models of NO biotransport in the microcirculation (reviewed by Buerk, Barbee, and Jaron,⁸⁰ in this issue). The models of NO , O_2^- , and ONOO^- interactions are not many, and the important results are summarized in this section.

Chavez et al.,⁸¹ Kavdia,⁸² and Buerk et al.⁸³ developed mathematical models of free radical transport in and around an arteriole. These models used detailed arteriolar vessel geometry and solved general mass transport equations for cylindrical geometry. The convective mass transport was neglected because of fast reactions of these radicals. Buerk et al.⁸³ investigated the effect of varying O_2^- production rates and SOD concentrations (0.02–20 μM). The O_2^- formation rate was assumed to be proportional to metabolism in the different tissue layers. Chavez et al.⁸¹ and Kavdia⁸² investigated the role of O_2^- and ONOO^- in inactivating vasoactive NO and the O_2^- formation rate was assumed to be a fraction of NO production rates. Chavez et al.⁸¹ studied the effect of isoforms of SOD and Kavdia⁸² studied a possible role of the location of oxidative stress in vascular tissue on these interactions.

These models predicted that the highest NO level is in the endothelium. The maximum ONOO^- concentration occurs in the smooth muscle or in the outer vascular wall. However, the model differed in the O_2^- concentration predictions. Chavez et al.⁸¹ and Kavdia⁸² reported that the maximum O_2^- concentration is in endothelial cells and is significantly higher compared with O_2^- concentrations in any other regions of the vascular wall or lumen,

whereas Buerk et al.⁸³ reported that the O_2^- concentrations can be significantly high in all regions of vascular wall. The difference in O_2^- concentrations are because of different O_2^- formation rates used by these groups. The important results from these studies are that (1) the range of concentration of NO, O_2^- , and ONOO⁻ are 50–150 nM, 0.1–300 pM, and 1–30 nM, respectively; (2) ONOO⁻ diffuses over a large distance, and (3) a reduction in SOD levels increases O_2^- and ONOO⁻ and decreases NO level.

These results indicate that the nitrosylation of membrane proteins and oxidization of lipids in the microcirculation may occur at diverse locations in diabetes, aging, and cardiovascular diseases. Because the location of oxidative stress vary greatly, a need for careful experiments at the individual vascular wall cell layer for oxidative stress studies in the vasculature is emphasized.⁸²

V. INTEGRATED EXPERIMENTAL AND COMPUTATIONAL MODELS

As described above, when NO, O_2^- , and ONOO⁻ are present in biological systems, their interaction leads to biologically significant outcomes including oxidation, nitration, and nitrosation. In biological systems, most of the methods used render nonquantitative and indirect measurements of these species. In this section, we review integrated experimental and computational models of dynamics of NO/ O_2^- /ONOO⁻ in systems including ischemia/reperfusion in rat hippocampus,⁴⁸ high glucose-exposed endothelial cells in parallel plate flow chamber,⁸⁴ and inactivation and nitration of SOD for varying fluxes of NO and O_2^- .⁸⁵ Note that the use of these integrated approaches leads to a quantitative understanding of complex interactions and concentrations of NO, O_2^- , and ONOO⁻ in these diverse biological systems using different approaches.

To understand ischemia/reperfusion-related NO and ONOO⁻ concentration dynamics, Yang et al.⁴⁸ measured NO concentration using a NO-selective sensor in the rat brain hippocampus during brain ischemia/reperfusion. The use of an integrated approach was to reveal ONOO⁻ dynamics. The chemical reaction network was composed of 7 reactions and 7 differential equations. The measured NO concentration and resulting NO formation rates were used to calculate the ONOO⁻ concentrations. The results indicated that during the reperfusion stage increased NO production rates can lead to a significant increase in ONOO⁻ levels.

Potdar and Kavdia⁸⁴ developed an integrated experimental and computation model to provide a quantitative understanding of interaction dynamics of NO/ O_2^- /ONOO⁻ in high glucose-induced endothelial dysfunction. They measured the widely used end-products of these interactions, nitrite and nitrate, using a chemiluminescence analyzer. The developed biochemical reaction network model was used to provide estimation of NO, O_2^- , and ONOO⁻ concentrations and productions from endothelial cells exposed to shear stress. The integrated approach revealed that both NO and O_2^- production of endothelial cells increased but NO bioavailability decreased upon exposure to high glucose. O_2^- and ONOO⁻ concentrations increased significantly upon high glucose exposure. This study emphasize the need for the recognition that there is a difference between production and level (or concentration) of these molecules and these cannot be used interchangeably, which is the

case in many experimental studies. In addition, the administration of SOD decreased O_2^- concentration and increased NO concentration. Importantly, this study provides an explanation for the contradictory results of vitamin-E supplementation clinical studies. The study reported that NO production was inhibited and O_2^- production decreased upon administration of vitamin E. An inhibition of NO production and subsequent decrease in NO bioavailability might be the possible cause for worsening of endothelial function in clinical studies of vitamin E supplementation.

Demicheli et al.⁸⁵ studied SOD inactivation by $ONOO^-$. The $ONOO^-$ formation was achieved using simultaneous fluxes of NO and O_2^- with varying ratios. Exposure of 5 μ M SOD to simultaneous equimolar flux of NO and O_2^- (10 μ M/min) led to inactivation of SOD in a time-dependent manner. The SOD inactivation was partially inhibited by glutathione but not by uric acid, a scavenger for $ONOO^-$ -derived radicals. They suggest that only $ONOO^-$, and not the products of hemolysis of $ONOO^-$, is responsible for inactivation of SOD. Using the integrated approach, the concentration of NO, O_2^- , and $ONOO^-$ were calculated under the experimental conditions. The NO and O_2^- concentrations are affected significantly by the presence of SOD. The O_2^- concentration changes from nanomolar to picomolar and NO concentrations changes from nanomolar to micromolar in the presence of SOD. However, $ONOO^-$ concentration decreases only ~37% and is capable of reacting with multiple targets.

VI. CONCLUSIONS

Even though great advances have been made in free radical measurements, the spatial and temporal resolution of the methods have not been sufficient to measure the steep gradients, transient intermediates, and in many cases very small concentrations of free radicals. There are significant advantages of computational modeling of NO, ROS, and RNS. The modeling of these molecules complex pathways (1) can provide information about intermediate products involved in a reaction, and (2) can determine the range of parameters for a specific result and help to elucidate the precise mechanism. Because most reaction constants in the models of ROS and RNS are obtained from precise, controlled reaction medium in isolation, it is vital that integrated experimental and computational modeling approaches continue to evolve for a given biological system and account for as many pathways as required to provide a better understanding.

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TABLE 1

Recent Literature Reviews of Nitric Oxide, Reactive Oxygen Species, and Reactive Nitrogen Species' Roles in Biological Systems

Review Topic	Reference
General health and disease	Pacher et al. (15)
Immune response	Wink et al. (86)
Molecular mechanism and therapeutic implications in heart failure	Nediani et al. (87)
Cytoneurotoxicity and neurologic disorders	Metodiewa and Koska (88)
Regulation of endothelial barrier function	Boueiz and Hassoun (89)
Intracellular signaling in skeletal muscle	Powers et al. (90)
Regulation of neutrophil function	Fialkow et al. (91)
Depolymerization of polysaccharides and its outcome	Duan and Kasper (92)