Causal Link Between Oxidative Stress, Inflammation, and Hypertension

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Hypertension (HTN) is a major cause of stroke, left ventricular hypertrophy, congestive heart failure, arteriosclerosis, end-stage renal disease, and peripheral vascular disease. Oxidative stress and its constant companion, inflammation, play a critical part in the pathogenesis of many acute and chronic illnesses including HTN and its long-term complications. There is compelling evidence that oxidative stress, inflammation, and HTN are involved in a self-perpetuating vicious cycle which, if not interrupted, culminates in progressive target organ injury and dysfunction. This article is intended to review the available evidence for the role of oxidative stress and inflammation in the pathogenesis of HTN. In addition, evidence will be presented to demonstrate the role of HTN in the pathogenesis of oxidative stress and inflammation. Finally, evidence for participation of tissue angiotensin system in the vicious cycle of oxidative stress, inflammation, and HTN will be presented, and the approach to treatment of HTN-associated oxidative stress will be discussed.

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INTRODUCTION

In recent years, compelling evidence has emerged pointing to the causal interconnection between oxidative stress, inflammation, and high arterial pressure in different forms of hereditary and acquired hypertension (HTN). This assertion is based on the following observations: first, oxidative stress is associated with elevated arterial pressure in nearly all animal models of HTN. Second, amelioration of oxidative stress reduces blood pressure in hypertensive animals. Third, induction of oxidative stress causes HTN in genetically normal, otherwise intact animals. Moreover, blockade of production of reactive oxygen species (ROS) attenuates pressor response to angiotensin II infusion. Fourth, oxidative stress and hypertension are accompanied by renal tubulointerstitial infiltration of T lymphocytes and macrophages. Finally, interventions aimed at preventing or reversing inflammation ameliorate oxidative stress and lower arterial pressure in hypertensive animals. This article is intended to provide a brief review of the nature, the mechanism, and the potential approach to management of oxidative stress in HTN.

FORMATION AND METABOLISM OF REACTIVE OXYGEN SPECIES

Under normal conditions, significant amounts of ROS such as superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) are produced in the course of oxygen metabolism. The primary sources of ROS include mitochondrial electron transport system and various oxidase enzymes including nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, cycloxygenase, lipoxygenase, P450 enzymes, glucose oxidase, and uncoupled nitric oxide synthases among others (Figure 1). The primary ROS produced in the body is superoxide which is generated from the 1-electron reduction

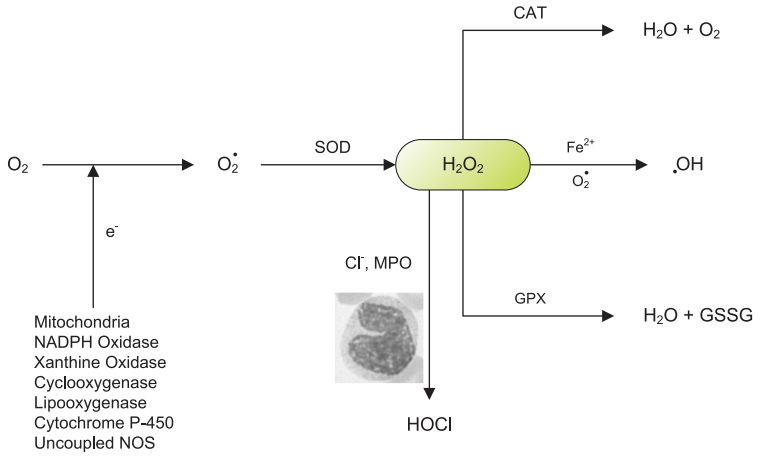

Figure 1. Production and metabolism of reactive oxygen species. NADPH indicates nicotinamide adenine dinucleotide phosphate; NOS, nitric oxide sythase; GSSG, glutathione disulfide; CAT, catalase; SOD, superoxide dismutase; GPX, glutathione peroxidase; and MPO, myeloperoxidase.

of molecular oxygen:

$O_2 + e^- \rightarrow O_2^{\bullet -}$

Superoxide is a short-lived, highly reactive, and potentially cytotoxic molecule which can attack, denature, or modify adjacent molecules. For instance, superoxide avidly reacts with and inactivates nitric oxide (NO), an event which leads to formation of peroxynitrite and reduction of bio-available NO:

$O_2^{\bullet -} + NO \rightarrow ONOO^-$

Peroxynitrite is an extremely reactive nitrogen species, which attacks, denature, and damages lipids, proteins, and nucleic acids. For instance, peroxynitrite reacts with tyrosine residues in protein molecules to produce nitrotyrosine. This phenomenon can alter function and structure of proteins and interfere with cellular signal transduction pathways involving tyrosine phosphorylation.

Tyrosine + $ONOO^-$ ⟶ Nitrotyrosine

Normally, superoxide is converted to H_2O_2 by a family of enzymes known as superoxide dismutase (SOD). Three different SOD isoforms have been, thus far, identified: cytoplasmic isoform (cuprozinc-containing SOD); mitochondrial isoform (manganese SOD), and extracellular SOD.

$O_2^{\bullet -} + O_2^{\bullet -} + 2H \xrightarrow{SOD} H_2O + O_2$

H_2O_2 is normally converted to water by either catalase (CAT) or glutathione peroxidase (GPX). Glutathione peroxidase uses reduced glutathione (GSH) as its substrate:

$2H_2O_2 \xrightarrow{CAT} H_2O + O_2$

$H_2O_2 + 2GSH \xrightarrow{GPX} 2H_2O + GS–SG$ (Oxidized Glutathione)

However, in the presence of electron donors such as iron (Fe^{2+}) and other transition metals (eg, Cu^{2+}) or superoxide ($O_2^{\bullet -}$), H_2O_2 is converted to hydroxyl radical (.OH), which is the most reactive cytotoxic radical known. Hydroxyl radical attacks and denatures the adjacent molecules such as lipids, proteins, carbohydrates, and nucleic acids (eg, DNA).

$H_2O_2 + Fe^{2+} \longrightarrow .OH + OH^- + Fe^{3+}$ (Fenton Reaction)

$H_2O_2 + O_2^{\bullet -} \longrightarrow .OH + OH^- + O_2$ (Haber Wiess Reaction)

In addition, in the presence of inflammation, phagocytes produce and convert H_2O_2 to hypochlorous acid via the enzyme, myeloperoxidase (MPO), which is highly abundant in these cells.

$H_2O_2 + Cl^- + H \xrightarrow{MPO} HOCl + H_2O$

Hypochlorous acid is a highly reactive chlorine species which can oxidize a variety of molecules, including proteins, to cause tissue injury and dysfunction. For instance, byproducts of MPO reactions are abundantly present in atherosclerosis plaques.

ANTIOXIDANT DEFENSE SYSTEM

Under normal conditions, ROS and the byproducts of their reactions with various biomolecules are neutralized and converted to harmless molecules by the natural antioxidant system. The antioxidant defense system is a highly complex biochemical organization that consists of numerous enzymes and a large number of scavenger molecules. Each

of these enzymes and antioxidant molecules participate in highly specific reactions and, as such, are not interchangeable. The body's pool of antioxidant molecules is derived from endogenous and exogenous sources. The exogenous antioxidants include molecules such as various vitamins and phytochemicals as well as byproducts of normal colonic microbial organisms.

Interaction of antioxidant molecules with ROS and other reactive species protects the functional and structural moles from oxidative damage. In this context, antioxidant molecules act as soldiers who protect civilian populations against invading enemies. It is of note that antioxidant molecules interacting with ROS become free radicals themselves and have to be neutralized by other specific enzymes or antioxidant molecules. Consequently, consumption of very high levels of any antioxidant can paradoxically initiate or exacerbate oxidative stress due to accumulation of its free radical metabolite. In fact, increasing number of cancer and cardiovascular disease prevention trials have demonstrated heightened rather than decreased risk with oversupplementation of various antioxidants. These observations highlight the importance of a well-balanced antioxidant system for maintenance of health and disease prevention.

OXIDATIVE STRESS

Oxidative stress is a consequence of the imbalance between ROS production and antioxidant capacity. This can occur as a result of either heightened ROS generation, impaired antioxidant system, or a combination of both. In the presence of oxidative stress, uncontained ROS attack, modify, and denature functional and structural molecules leading to tissue injury and dysfunction. I wish to point out that while excessive production of ROS causes injury and dysfunction, normal rate of ROS production is essential for life. This is because ROS serve many biologically important roles in signal transduction, regulation of cell growth and apoptosis, fetal development, and innate immunity, among other functions.

Evidence for Causal Role of Oxidative Stress in Hypertension

Increasing evidence has emerged that point to a causal link between oxidative stress, HTN, and inflammation.[1,2] This proposition is based on the following observations:

First, a consistent association has been found between HTN and oxidative stress in the kidney, blood vessels, and brain in nearly all forms of acquired and hereditary HTN in experimental animals. For instance, oxidative stress has been shown to be present in animals with HTN caused by chronic lead exposure, chronic kidney disease, deoxycorticosterone acetate-salt administration, aorta coarctation, diabetes mellitus, metabolic syndrome, nitric oxide synthase (NOS) inhibition, high salt intake, and angiotensin II.[3-34]

Second, alleviation of oxidative stress with pharmacological doses of several antioxidants has been shown to reduce blood pressure in hypertensive animals, but not in the normotensive animals.[3,4,6-11,15-24]

And third, the observations cited above represent indirect evidence for the role of oxidative stress in the pathogenesis of HTN. Direct evidence for the causal role of oxidative stress comes from the following observations: (a) induction of oxidative stress has been shown to cause HTN in genetically normal, otherwise intact, animals[35,36]; (b) mice with manganese SOD deficiency exhibit salt-sensitive HTN[37]; and (c) binding of angiotensin II to the angiotensin I (AT1) receptor results in production of ROS via activation of NADPH oxidase in the kidney and vasculature. Activation of NADPH oxidase involves assembly of the enzyme's cytoplasmic ($P47^{phox}$ and $P67^{phox}$) and membrane-associated ($p22^{phox}$ and $gp91^{phox}$ or its tissue-specific isoforms, NOX-1, NOX-4, etc) subunits. The ROS production and hypertensive response to angiotensin II infusion is attenuated by pharmacological inhibition of NADPH oxidase and by suppression of its $p22^{phox}$ subunit expression.[38,39] These observations illustrate the role of ROS as a major mediator of the pressor action of angiotensin II.

Evidence for the Role of Hypertension as a Cause of Oxidative Stress

The observations cited above provide irrefutable evidence that oxidative stress in the kidney, blood vessels, and brain causes HTN. Conversely, HTN, per se, has been shown to cause oxidative stress. This assertion is based on investigations that revealed presence of oxidative stress in the vascular tree residing

proximal to (hypertensive zone), but not distal to the abdominal aorta coarctation in rats with abdominal aorta banding.[40-42] Since both of the arterial segments are supplied by the same blood in this model, these experiments clearly illustrate the role of high blood pressure and shear stress as opposed to those of circulating hormones and other humoral factors as a cause of oxidative stress. Taken together, these observations suggest that oxidative stress can cause HTN, and HTN can cause oxidative stress; hence, the two conditions are involved in a self-perpetuating cycle.

Cellular and Molecular Sources of Oxidative Stress in Hypertension

Oxidative stress is a condition in which generation of reactive oxygen species (ROS) exceeds the capacity of the antioxidant defense system. Thus, oxidative stress can occur as a consequence of excess generation of ROS, depressed antioxidant capacity, or a combination thereof.

The NADPH oxidase family of enzymes has been identified as the main source of ROS in the kidney and vascular tissues in various models of HTN.[43] This enzyme was originally found in phagocytes serving as a source of ROS to destroy invading microbes. More recently, NADPH oxidase and its closely related isotypes have been found in numerous other cell types including endothelial cells, renal tubular epithelial cells, and vascular smooth muscle cells (NOX-1 and NOX-4). Shear stress, angiotensin II, and proinflammatory cytokines which are intimately related to HTN can activate and/or upregulate NADPH oxidases. In fact, upregulation of NADPH oxidase and its isotypes has been demonstrated in various models of HTN.[12,19,25,32]

Insufficient Antioxidant System

Although excessive production of ROS is the most common cause of oxidative stress in HTN, it is occasionally caused by primary impairment of antioxidant system. For instance, hereditary mitochondrial SOD deficiency causes salt-sensitive HTN in mice,[37] and glutathione depletion can raise blood pressure in rats.[35,36] Moreover, persistent oxidative stress can deplete antioxidant molecules and inactivate antioxidant enzymes and, thereby, impair antioxidant defense system. In fact, several recent studies have demonstrated significant impairment of antioxidant enzymes in various models of HTN including spontaneously hypertensive rats and rats with chronic kidney failure, lead-induced HTN, diabetes, and salt-sensitive Dahl rats.[6,12,16,32]

Immune Cell Activation in Hypertension

Several studies have demonstrated renal tubulointerstitial infiltration of activated macrophages and T lymphocytes in various animal models of HTN.[23,24,32,44-48] These findings point to the association of HTN with inflammation. The infiltrating immune cells, as well as cells of renal origin, have been shown to produce superoxide and express angiotensin II, events that can contribute to oxidative stress and HTN.[23,24] This assumption is supported by the observations that interventions aimed at reducing the inflammatory infiltrate result in amelioration of HTN.[45-48] It is of note that the activated immune cells release large quantities of ROS which promotes regional oxidative stress. Conversely, oxidative stress promotes inflammation by activating the redox-sensitive transcription factor, nuclear factor-kappa B (NF-kappa B) which, in turn, triggers generation of proinflammatory cytokines and chemokines, and hence, inflammation (Figure 2).

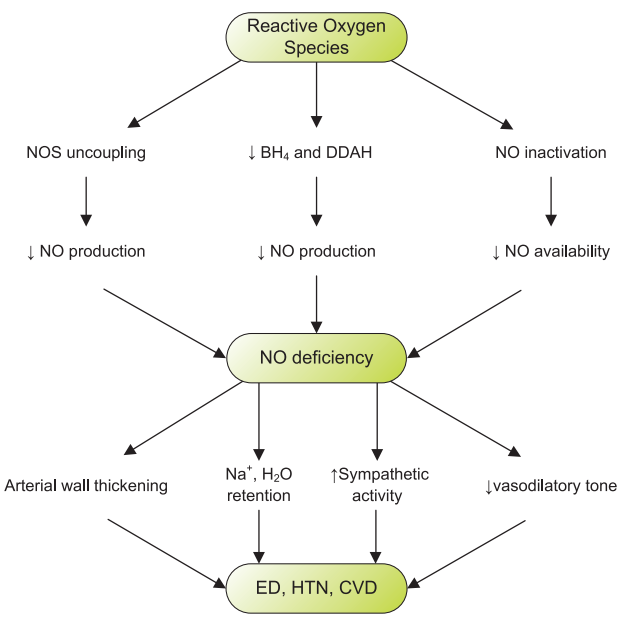

Figure 2. Effects of oxidative stress on nitric oxide metabolism and action. NO indicates nitric oxide; BH4, tetrahydrobiopterin; DDAH, dimethyl arginine dimethyl aminohydrolase; NOS, nitric oxide sythase; ED, endothelial dysfunction; and CVD, cardiovascular disease.

This supposition is supported by recent studies that clearly demonstrated concurrent NF-kappa B activation and tubulointerstitial inflammation and their amelioration with antioxidant therapy in hypertensive animals.[23,24]

Taken together, these studies have identified renal parenchymal cells, resident macrophages, and infiltrating inflammatory cells as the source of ROS in the kidney of hypertensive animals. In addition, generation of ROS by endothelial cells, vascular smooth muscle cells, and circulating leukocytes contribute to oxidative stress in the vascular tissue.

Mechanisms by Which Oxidative Stress Increases Blood Pressure

Oxidative stress can raise blood pressure by several mechanisms:

(1) Oxidative stress limits bioavailability of NO in key tissues and organs involved in blood pressure regulation by several mechanisms (Figure 3): first, ROS avidly react with and inactivate NO. Second, ROS reduce NO production by uncoupling endothelial NO sythase (eNOS), by depleting tetrahydrobiopterin, which is the NOS cofactor, and by promoting accumulation of asymmetrical dimethyl-arginine (ADMA), which is a potent endogenous NOS inhibitor. The latter is caused by ROS-mediated inhibition of dimethylarginin dimethyl-aminohydrolase (the enzyme that metabolizes ADMA) and by upregulating the enzyme, protein methyl-transferase-1 which catalyzes arginine methylation, and hence, generation of ADMA.[49,50] The reduction of NO availability by oxidative stress in the vascular tissue can raise systemic vascular resistance, and hence, blood pressure by lowering the NO-mediated vasodilatory tone. In addition, limitation of NO availability in the kidney can augment renal vascular resistance, increase tubular sodium and water reabsorption, and inhibit pressure natriuresis, events that can raise blood pressure via extracellular volume expansion. Moreover, diminished NO in the brain can increase central sympathetic outflow which can contribute to the rise in blood pressure.[51] Finally, oxidative stress can directly and indirectly (via lowering NO) promote endothelial dysfunction, vascular remodeling (matrix protein accumulation; vascular smooth muscle; and fibroblast migration, transformation, and proliferation), and leukocyte/platelet adhesion, events that lead to maintenance of HTN and progressive arteriosclerosis, atherosclerosis, and thrombosis.

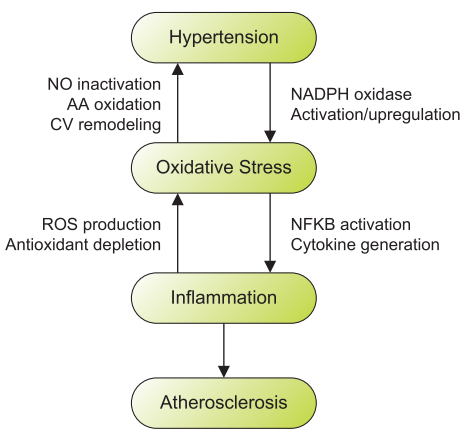

Figure 3. Interconnection of hypertension, oxidative stress, inflammation, and atherosclerosis. AA indicates arachidonic acid; CV, cardiovascular; ROS, reactive oxygen species; NFKB, nuclear factor-kappa B; NADPH, nicotinamide adenine dinucleotide phosphate; and NO, nitric oxide.

(2) The ROS result in nonenzymatic oxidation of arachidonic acid in lipoproteins and cell membrane phospholipids, which leads to generation of vasoconstrictive proinflammatory products such as isoprostanes. These byproducts of arachidonic acid oxidation can contribute to the rise in blood pressure and renal and cardiovascular complications.[18]

(3) The ROS can increase vascular smooth muscle tone by increasing cytoplasmic ionized calcium concentration ($[Ca^{2+}]_i$).[52]

(4) Oxidative stress can promote endothelial injury and dysfunction which can support development of HTN and cardiovascular disease.

ROLE OF INFLAMMATION IN PATHOGENESIS OF HYPERTENSION

Figures 3 and 4 show the links between oxidative stress, inflammation, and HTN. There is increasing evidence supporting the role of renal tubulointerstitial and vascular inflammation in the pathogenesis of HTN.[1,53] In fact, renal tubulointerstitial infiltration of T lymphocytes and macrophages has been shown in essentially all animal models of hereditary and acquired HTN. Renal tubulointerstitial inflammation is accompanied by activation of NF-kappa B,[32,44,54] which is the general transcriptional factor for

many proinflammatory cytokines, chemokines, and adhesion molecules.

In addition, several studies have demonstrated activation of circulating leukocytes in hypertensive humans and animals.[55-62] The causal role of inflammation in the pathogenesis of HTN is supported by a number of animal studies that have shown amelioration of HTN with interventions aimed at blocking inflammation including the use of NF-kappa B activation inhibitor,[63,64] and the immunosuppressive drug, mycophenolate mofetil.[48,51,65]

It is of note that inflammation and oxidative stress are inseparably interconnected (Figure 4). For instance, by activating NF-kappa B and activator protein-1, oxidative stress stimulates production of chemokines, cytokines, and adhesion molecules as well as activation and proliferation of lymphocytes. These events, in turn, result in immune cell activation, adhesion, and infiltration. Conversely, inflammation causes oxidative stress since production of the ROS is an inherent property of activated immune cells. Thus, oxidative stress and inflammation are involved in a self-perpetuating cycle. In fact, circulating blood leukocytes and immune cells infiltrating the kidney have been shown to produce ROS in hypertensive animals and humans.[24,59-62]

PARTICIPATION OF TISSUE ANGIOTENSIN SYSTEM

Activation of AT1 receptor by angiotensin II results in activation and upregulation of NADPH oxidase isoforms, and thereby, generation of ROS in the kidney and cardiovascular tissues.[66-68] The ROS produced in this manner promotes inflammation by activating NF-kappa B.[69,70] Angiotensin II has been shown to promote NF-kappa B activation in renal and vascular cells which leads to inflammation in these tissues.[71] Conversely, activation of NF-kappa B stimulates gene expression of angiotensinogen.[72] Accordingly, activations of tissue angiotensin system and NF-kappa B appear to be involved in a vicious cycle that contributes to HTN, renal injury, and inflammation. Thus, in addition to stimulating salt retention (directly in proximal tubules and indirectly via aldosterone in distal tubules) and vasoconstriction, angiotensin II promotes oxidative stress and inflammation. Conversely, inflammation raises renal tissue angiotensin system (Figure 5). This assertion is based on the observation that inflammatory cells constitute close to 50% of angiotensin II-expressing cells in hypertensive kidney.[53]

Several animal studies have shown marked upregulation of AT1 receptor and significant increase in the number of angiotensin II positive

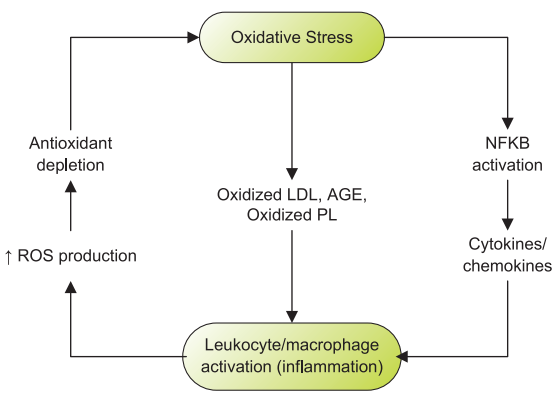

Figure 4. Link between oxidative stress and inflammation. LDL indicates low-density lipoprotein; PL, phospholipids; ROS, reactive oxygen species; NFKB, nuclear factor-kappa B; and AGE, advanced glycol-oxidation end products.

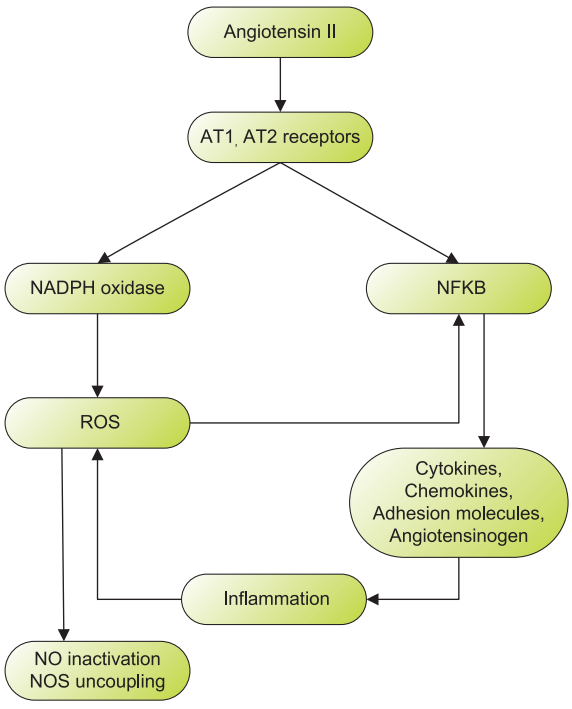

Figure 5. Role of angiotensin system in the pathogenesis of oxidative stress and inflammation. AT indicates angiotensin; ROS, reactive oxygen species; NFKB, nuclear factor-kappa B; NADPH, nicotinamide adenine dinucleotide phosphate; and NOS, nitric oxide synthase.

cells in the kidneys of hypertensive animals. Thus, tissue angiotensin system appears to be upregulated in many hypertensive disorders regardless of plasma renin levels.[32,44,73-76] These observations illustrate the interconnection of tissue angiotensin system with oxidative stress, inflammation, and hypertension and provide the rationale for renin-angiotensin system blockade in the management of hypertension.

TREATMENT OF OXIDATIVE STRESS IN HYPERTENSION

Successful management of oxidative stress in a given condition requires in-depth understanding of its cellular and biochemical mechanisms. Consequently, a mere administration of one or more antioxidant vitamins cannot cure oxidative stress in HTN, renal disease, diabetes, or other conditions. Instead, specific interventions directed at the specific underlying factor would be most effective. For instance, since HTN can cause oxidative stress, therapeutic interventions that can reduce blood pressure represent an ideal antioxidant therapy for the HTN-associated oxidative stress. In addition, since stimulation of AT1 receptors by angiotensin II promotes oxidative stress and HTN via activation and upregulation of NADPH oxidases, drugs that interrupt rennin-angiotensin system can be considered as specific therapies for management of oxidative stress in certain types of HTN, especially chronic kidney disease and diabetes mellitus. Similarly, adequate glycemia control in diabetes and lipid-lowering strategies in hyperlipidemia are most effective in reversing oxidative stress associated with these conditions. Finally, consumption of a diet rich in natural antioxidants and other essential micronutrients (present in fresh fruits, vegetables, and nuts), as well as regular exercise and weight control, would be desirable in combating oxidative stress and promoting good health.[13,77]

Not surprisingly, cardiovascular and cancer prevention trials of high doses of several antioxidant compounds including tocopherol, beta carotene, ascorbic acid, selenium, and other agents have shown no benefit or increased instead of decreased risk.[78-83] As noted in a recent review,[1] and summarized in the Table, several factors contribute to the lack of benefit and potential adverse effects of high doses of antioxidant agents. First, since oxidative stress in HTN is not caused by deficiency of the given antioxidants, it cannot be corrected by administration of such agents. Second, administration of supraphysiologic quantities of the given antioxidant compounds would lead to accumulation of their free radical metabolite (for example, ascorbyl radical with vitamin C, tocopheroxyl radical with vitamin E, etc) which can actually worsen oxidative stress. For these and other reasons listed in the Table, consumption of high doses of these agents is not recommended for the treatment of HTN and cardiovascular disease.

CONFLICT OF INTEREST

None declared.

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Possible Explanations for Failure of Clinical Trials on Antioxidant Agents for Treatment of Hypertension

The given antioxidant (eg, vitamin E) is unable to prevent interaction of ROS (eg, HOCl, .OH, and ONOO) with target molecules
The given antioxidant is unable to reach the reaction site (eg, lipid versus aqueous phase).
Processing large quantities of free radical form of the given antioxidant is not possible (intensification of oxidative stress by iatrogenic imbalance in antioxidant system).
Since oxidative stress is primarily caused by excess ROS production, antioxidant administration merely represents a symptomatic rather than curative approach in the treatment of oxidative stress in HTN and cardiovascular disease.
Antioxidant system is not designed to abolish inflammation-induced oxidative stress which is an essential component of the innate immune response.

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