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Oxidative stress in hypertension

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Abstract

Reactive oxygen species interfere with the mechanisms controlling BP and play an important role in the development of hypertension and vascular damage. Oxidative stress is increased in hypertensive subjects even in cells other than those present in the vascular wall. This increased oxidative stress, not related to BP values, is accompanied by a reduction in the most important antioxidant mechanisms and by an accumulation of reactive oxygen species byproducts, not only from lipid peroxidation but also from oxidized genomic and mitochondrial DNA. To better understand the clinical significance of oxidative stress in hypertension, it is necessary to explore whether the reduction in antioxidant mechanisms is the cause or the consequence of oxidative stress.

Keywords: Hypertension; Oxidative stress; Blood pressure.

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Introduction

Since the discovery of superoxide dismutase (SOD) by Fridowich and McCord, it has become clear that highly reactive oxygen free radicals are formed inside cells as a result of aerobic metabolism. Free radical-induced oxidative stress has emerged as a valuable hypothesis to explain many pathological states. Aging and a large number of pathophysiological syndromes, including inflammation and other associated degenerative processes, are recognised as depending on the unbalance of the redox equilibrium between prooxidants and antioxidants in the intracellular millieu[1-3].

Oxidative stress is the result of the overproduction of reactive oxygen species (ROS), including superoxide ions (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (-OH)[4]. An increase in ROS is the result of many different sources and mechanisms, including radiation exposure, xenobiotic overdose, inflammatory reactions, spontaneous autoxidations and environmental contamination. Under these circumstances, antioxidant mechanisms such as superoxide dismutase (SOD), catalase and glutathione perodase (GPx) and other free radical scavengers, vitamin E, vitamin C, and reduced glutathione (GSH), may be overwhelmed. Consequently, ROS easily reacts with target biomolecules, thus leading to

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metabolic dysfunctions and cell molecule oxidation[5].

The biological significance of oxidative stress could be interpreted in two different ways. First, some of these radicals, such as superoxide, may have regulatory properties under physiological circumstances, thereby controlling metabolic processes and gene expression. Second, ROS are very reactive molecules which could induce cell and tissue damage by reacting with different molecules in a cytotoxic manner in which lipids, proteins, carbohydrates and nucleic acids are damaged by the oxidation process[3,5-6] (Figure 1). Lipid peroxidation represents one of the most clear consequences of free radical attacks on a biological systems. Oxidation of membrane phospholipids may lead to an alteration cell viability, to a distortion of signal transduction pathways, and to an oxidation of plasma lipoproteins such as LDL which is known to increase the lipoprotein atherogenic potential[7]. Oxidative DNA modification may have profound implications in tumor initiation and development[8]. At the protein level, free radicals induce changes and serious repercussions on protein turnover, enzyme and transcription factor activities. Therefore, oxidative stress may be not only the cause but also the consequence of many cell and tissue disturbances[3].

During the past few years, different reports have suggested that free radical production underlies the physiopathological mechanism of hypertension (HTN) and HTN-induced organ damage, proposing an antioxidant intervention to ameliorate or prevent its clinical complications. Strategies focused on combating hypertension and vascular diseases through the inhibition of superoxide-generating enzymes have been proposed. While results from animal studies are promising, no consensus has yet been reached in human hypertension.

In the present issue, we focus our interest on the role of oxidative stress in hypertension (HTN), reviewing the most relevant data obtained from both experimental and clinical studies.

Oxidative stress, angiotensin II and nitric oxide

Angiotensin II (ANGII) and nitric oxide (NO) are two of the most important molecules underlying cardiovascular system regulation, and their central role in the development of HTN and HTN-induced organ damage have been firmly established. The interrelations between these two molecules and ROS have received increasing attention in the last few years. As a result, recent hypotheses have proposed that ANGII is responsible not only for elevated blood pressure but also for the oxidative stress which accompanies hypertension[9]. In turn, most of the available experimental data proposes the inactivation of the vasodilator agent nitric oxide (NO) by ROS as a crucial event in raising blood pressure.

Enhanced ROS-induced oxidative stress, which is mainly mediated by superoxide and hydroxyl radicals, occurs in human hypertension and a wide variety of animal models which include spontaneous hypertension rats[10], renovascular hypertension[11], deoxycorticosterone acetate-salt model[12], and obesity-related hypertension[13]. Superoxide, one of the most active ROS in the vascular wall, is produced by the activity of NADPH oxidase, xanthine oxidase and uncoupled nitric oxide synthase.

Nitric oxide is synthesised by at least three known enzymes, and the isoform expressed in endothelial cells (eNOS) appears to play a major role in blood pressure regulation. This isoform is activated by elevations of intracellular Ca^{++} acting through calmodulin. At the biochemical level, the effect of NO on smooth muscle appears to be mediated by a soluble form of guanylyl cyclase that contains heme bound in a pentacoordinate manner. As a consequence of this NO-guanylyl cyclase interaction, cyclic GMP production is over-stimulated, which in turn results in the activation of a G-kinase I (cGKI isoform) which is required for cyclic GMP action on smooth muscle. In the kidney, the G-kinase II appears to function as a component of a renin release regulatory pathway[14].

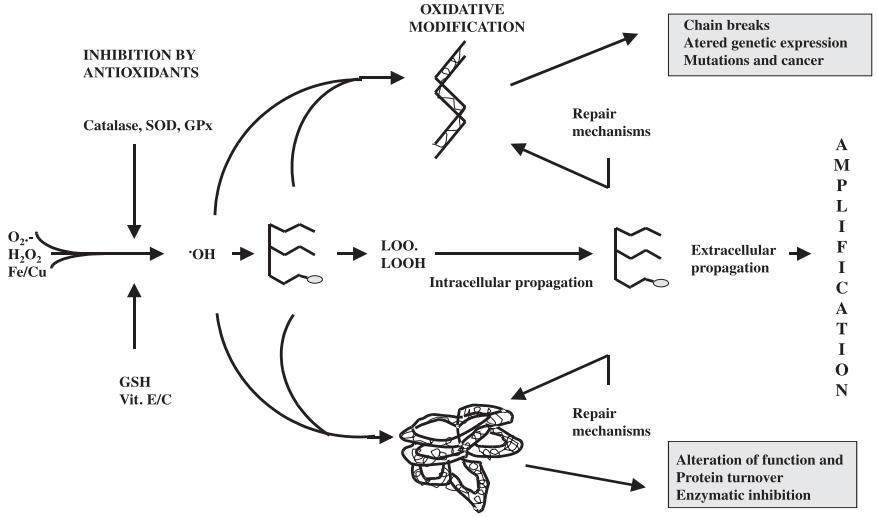

Figure 1 – Interaction of ROS with maromolecules.

Nitric oxide inactivation has been attributed to an increase of NADH/NADPH oxidase activity leading to superoxide production in rats[15], and ANGII has been shown to stimulate the production of superoxide[16]. At the same time, a high affinity chemical combination of superoxide with NO is known to yield peroxinitrite in a constant rate reaction similar to that of the superoxide for SOD or NO for heme compounds[9]. Peroxinitrite is a potent oxidant that could oxidize arachidonic acid and release a potent renal vasoconstrictor and antinatriuretic substance, 8-iso-prostaglandin $F_{2\alpha}$ (isoprostane)[17]. Moreover, peroxinitrite has been shown to upregulate the cyclooxygenase-mediated production of prostaglandin E2 in macrophages from old mice, and can inhibit prostacyclin synthesis activity, leading to a reduction of prostacyclin in endothelial cells[9]. Furthermore, peroxinitrous acid is a constant source of active hydroxyl radicals[17].

The mechanism by which ANGII induces the production of superoxide ions has not been clearly elucidated. ANGII infusion increases the expression of nox 1, gp91(phox), and p22(phox), all of them subunits of NADPH oxidase, by a protein kinase C (PCK) mechanism[18]. Several investigators have shown that membrane-associated NADPH oxidases are the primary physiological producers of ROS in vascular tissue. All subunits of NADPH oxidase, however, are not equally distributed in vascular cells. This is the case for gp91phox, which is absent in smooth muscle cells (SMC) suggesting that a substitute must exist. Recently, several homologues of gp91phox have been cloned, and one of them, termed mox-1 for mitogenic oxidase, also known as nox-1, has been shown to be expressed in vascular smooth muscle cells. In these cells, nox-1 antisense attenuates O_2^- production in response to platelet-derived growth factor (PDGF).

Besides the ROS-induced reduction of NO bioviability, ROS can also reduce NO synthesis. Under oxidative stress conditions, tetrahydropterin (BH4), a cofactor of NO synthases, can be oxidized to dihydropterin (BH2). As a result, the enzyme becomes uncoupled producing O_2^- rather than NO[19,20]. Using both mice with a deficiency in the NAPPH oxidase subunit p47phox, as well as mice lacking either the endothelial or neuronal NO synthase it has been demonstrated that hypertension produces a cascade involving the production of ROS from the NADPH oxidase, leading to oxidation of tetrahydrobiopterin and uncoupling endothelial NO synthase. Furthermore, treatment of mice with oral tetrahydrobiopterin reduces vascular ROS production, increases NO production and blunts the increase of blood pressure in a DOCA-salt model of hypertension[21].

Reactive oxygen species regulate the expression of different classes of genes. Some of them are clearly an adaptive response, such as the induction of SOD by O_2^- and or catalase by H_2O_2[22]. Other examples of ROS-induced redox enzyme expression are manganese superoxide dismutase (MnSOD)[23], GPx[22], catalase[22] and heme oxigenase-1[24] (Table 1). Thus, an increase in the production of O_2^- by vascular and circulating cells as a result of an increase of NADPH oxidase activity may in turn increase the production of H_2O_2 by SOD-induced synthesis. If the availability of these two reactive species is above and beyond the capability of catalase and/or GPx, the highly reactive radical -OH may be formed via the Haver-Weiss or the Fenton reaction[1,3].

Different studies have shown a close relationship between the hypertensive effect of ANGII, ROS production and NO activity. The inhibition of NO synthesis enhances the vasoconstrictor effect of ANGII. Kimoto et al. found that the continuous administration of the specific NO synthesis inhibitor, L-NAME, to Sprague-Dawley rats for 7 days induces ROS, and this was blocked by ANGII receptor antagonists[25]. These authors found that the increase of ROS by L-NAME treatment was also blocked by antioxidant administration. Thus, it was suggested that a simple decrease in NO synthesis leaves unbalanced ANGII, which induces ROS production[26].

In addition to ANGII, other agonists and mechanical factors are able to induce ROS formation in vascular cells. Platelet-derived growth factor, thrombin, tumoral necrosis factor alpha (TNFα), and lactosylceramide activate NADPH oxidase-dependent O_2^- production in SMCs. Fibroblasts exhibit increased NADH

Table 1 – Redox proteins expression by redox modulation

Gene	Experimental model	Stimulus	Reference
Cu/Zn-SOD	Endothelial cells	H_2O_2	17
Mn-SOD	Endothelial cells	H_2O_2	18
Catalase	Endothelial cells	H_2O_2	17
GSH peroxidase	Endothelial cells	H_2O_2	17
Heme oxigenase-1	Endothelial cells	H_2O_2	19

or NADPH-driven O_2^- production in response to TNFα, IL-1 and PDGF. In endothelial cells mechanical forces, including cyclic stretch and oscillatory shear stress, stimulate NADPH oxidase activity. The precise signals responsible for oxidative activation by each of these stimuli remain to be established (reference 27 is a review) (Figure 2).

Oxidative stress and blood pressure regulation

The role of oxidative stress in the complex mechanismis involved in BP control has been extensively analyzed. Interaction of ROS with ANGII and NO or with other mediators influences not only the remodelling process of the vascular wall, and as a consequence peripheral resistance, but also the cardiac contractibility, barroreceptor sensitivity, adrenergic activity and sodium handling mechanisms in the kidney.

Endothelial dysfunction is one of the main consequences of the accelerated inactivation of NO by ROS. As a consequence of the endothelial dysfunction, an increase in arterial wall remodelling, platelet aggregation and inflamation, and smooth muscle cell proliferation and vascoconstriction have been described. All of these consequences contribute to rising BP values and to develop in the arterial wall lesions of atherosclerosis.

In addition to the vascular action of oxidative stress, evidence is emerging that increased ROS in the kidney might also contribute to the genesis and development of HTN by interfering with sodium handling. In spontaneous hypertensive rats (SHR) a reduced bioavailability of NO in the macula densa, as a consequence of ROS, favouring vasoconstriction and enhancing the tubular glomerular feedback response has been described. The antihypertensive activity of TEMPOL in this model has been shown to be associated with a selective increase in medullary blood flow and a reduction of the renal medullary vasoconstrictor effects of angiotensin II[28]. In Dahl S rats exposed to 3 weeks of high salt diet there is a reduced renal SOD activity and elevated urinary $F_2\alpha$ isoprostanes indicating a state of increased oxidative stress in the kidney of these hypertensive rats[29]. Furthermore, infusion of a SOD-inhibitor, DETC, markedly reduce renal medullar blood flow and sodium excretion.

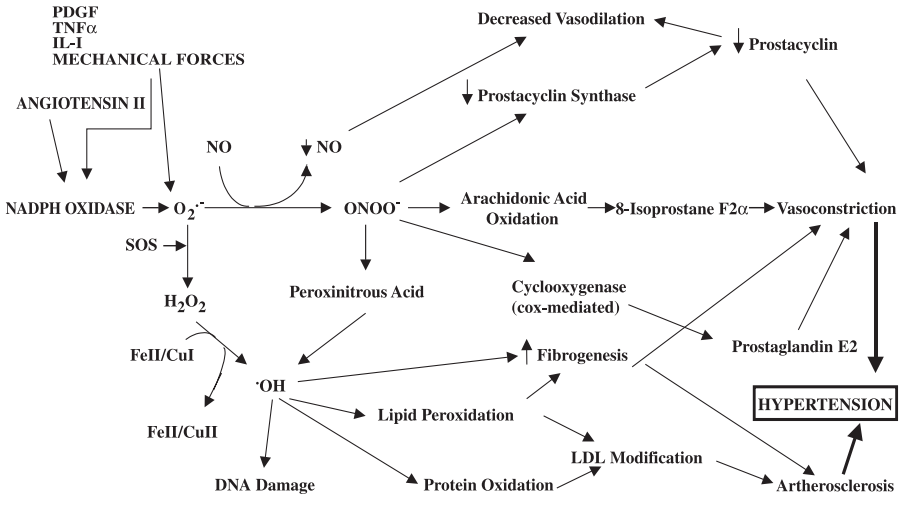

Figure 2 – Ischematic representation of oxidative mechanisms in hypertension.

Thus, an increment in superoxide in the renal medulla might be an important pathogenic mechanism resulting from HTN. Recent research seems to point to the accumulation of H_2O_2 in the renal medulla as a contribut to the hypertensive consequences of oxidative stress[30]. The fact that the results of a salt-sensitive model of HTN show either a reduction of NO synthesis or an induction of ROS production, points to the necessity of a thigh balance between the mechanism increasing or decreasing NO bioavailability in salt reabsorption.

A ROS-induced autonomic dysregulation has also been demonstrated. An increase in sympathetic activity and decreased parasympathetic activity, barroreceptor sensitivity and heart rate variability are phenomenona which have been observed in several experimental models of HTN. The contribution of ROS to the hypertensive process is less well known than the previous outlined ones[31-32].

Antioxidants and hypertension

In different experimental models of hypertension, alterations of antioxidant enzymes have been demonstrated and correlated with an increase in ROS production. Both in spontaneously hypertensive rats (SHR) and in hypertensive subjects, decreased activity of antioxidant enzymes has been reported. Antioxidant molecules such as reduced glutathione (GSH) are also reduced in the circulating cells of hypertensive subjects. Moreover, an increase in the concentration of GSSG, the oxidized form of gluthation, in the blood of hypertensive patients, suggests that oxidative stress is present in human hypertension[33].

A decrease in SOD activity was found in patients with uncontrolled

hypertension. In a hypertensive tibetan population, the activity of SOD and total antioxidant capacity were found to be significantly lower compared to controlled normotensive subjects. In the patient group the increase of MDA was strongly correlated with the decrease of blood antioxidant content and the reduction of NO synthesis[34]. Recently, in a Spanish community of hypertensive subjects, a positive significant correlation has been found between levels of MDA and 8-oxo-dG in circulating cells[33].

The concentration of vitamin E and SOD were found to be decreased in leukocytes of Indian patients with uncontrolled hypertension[35]. The authors propose that an increase of ROS can inactivate prostacyclin and NO and decrease their half life which can lead to an increase in peripheral vascular resistance and hypertension.

If superoxide production is a common feature of experimental and human hypertension, decreasing radical levels by antioxidants might be expected to lower blood pressure values. A considerable number of experimental studies have demonstrated that an increase of SOD reduces arterial pressure in SHR[36].

In some, but not all studies, antioxidants appear to attenuate hypertension. The explanation for variability in the effects of antioxidants on blood pressure is not clear, but this variability may be due to several factors, including differences in cellular binding and/or cell permeability of antioxidants. Thus, intravenous administration of Cu/ZnSOD, which does not bind to cells and is not able to cross the cell membrane, does not reduce blood pressure in SHR[37] in angiotensin II-induced hypertensive rats[38] or in patients with essential hypertension[39]. Using different strategies in order to bind SOD to cells or using cell-permeable liposomes or a cell-permeable SOD mimetic[40,41] reduces blood pressure in experimental animals with hypertension. Chu et al. concluded that the heparin binding domain of extracellular SOD (ECSOD) is necessary to induce a reduction in blood pressure. They also observed that ECSOD did not reduce cardiac output in SHR, therefore, the reduction of blood pressure by ECSOD was attributed to a decrease of systemic vascular resistance[36].

In addition, Pedro-Botet et al. reported that in normolipidemic untreated mild hypertensive patients, SOD and Gpx activities are significantly decreased when compared with age-matched healthy controls[42]. In contrast to our recent observation[33], however, the group found a negative correlation between long transformed SOD activity and systolic and diastolic blood pressure ($r = 0.37$, $P < 0.0$; $r = 0.64$, $P < 0.0001$, respectively). They concluded that the low endogenous antioxidant enzyme activities observed may be in turn, a result of a decreased superoxide anion removal leading to nitric oxide inactivation[42].

Alterations of other antioxidant enzymes are also implicated in the mechanism of hypertension. This is the case of Catalase, an important enzyme in counteracting free radical toxicity. By converting H_2O_2 into oxygen and water, Catalase limits the deleterious effects of ROS. It has been found that genetic variation in Catalase is associated with essential hypertension[43]. The genetic predisposition of hypertension is, indeed, an important factor to be researched. Relevant observations have been reported regarding the linkage of gene polymorphism or mutation with human hypertension syndromes[44]. In SHRSP, which is considered an ideal model of human essential hypertension, a number of reproducible blood pressure regulation quantitative trait loci have been found to map rat chromosome 2. Genome-wide microarray expression profiling has allowed the identification of a significant reduction in the expression of glutathione S-transferase µ-type 2, a gene involved in the defence against oxidative stress[45]. With the emerging of new technologies to search the expression of a wide number of genes, it may be expected that the regulation of other antioxidant enzymes completes the genetic map of human hypertension.

Non-enzymatic proteins, antioxidant vitamins and/or other natural compounds are potent ROS scavengers. Additionally they have been found to play an important role in blood pressure regulation. Indeed, SOD decreases in uncontrolled hypertension are usually accompanied by low levels of antioxidant vitamins such as alpha-tocopherol[36]. An accumulating body of evidence has also demonstrated that antioxidant vitamins C and E are also implicated in the improvement of endothelial function in human subjects and in animal models of human disease including hypertension[46-48]. Clinical trials and oxidation studies show evidence suggesting that 100-400 IU of daily vitamin E over 2 years or more may be the most effective method of reducing low-density lipoprotein oxidation and positively influence mortality rates from cardiovascular disease in primary care[49]. Research also supports vitamin E supplementation in patients with coronary artery disease. Studies have revealed that hypertensive patients have a higher than normal susceptibility to LDL oxidation. It has been demonstrated that the daily administration of vitamin E (400 IU) reduces the susceptibility to oxidative modification of LDL and, therefore,

may prevent the increased risk of cardiovascular disease[50]. The intake of vitamin C has also been recommended, especially for people with hypertension and/or diabetes although evidence supporting the purported benefits is lacking[49].

Treatment of stroke-prone spontaneous hypertensive rats (SHRSP) with antioxidant vitamins C and E results in an increase of SOD activity and plasma total antioxidant status (TAS) and in a decrease of NADPH oxidase[48]. Vitamin C alone or combined with vitamin E has been shown to enhance NO generation and to reduce blood pressure in hypertensive animals[51]. In addition, vitamin E and C have been shown to protect endothelium dysfunction in SHR and to reduce superoxide production[52].

Additionally potent antioxidant molecules such as reduced glutathione (GSH) and thiol compounds, in general, play an important role in the regulation of endothelial tone directly or as a consequence of ROS reduction. By itself, GSH, may react with O_2^- and -OH radicals[53-54], the tripeptide being not only an efficient ROS scavenger but also a key regulator of a variety of metabolic and gene expression processes[55-56]. The reduction of GSH in hypertension is, again, an indication of free radical production and the secondary oxidative stress which characterises hypertension. Reactive oxygen species which are increased in hypertension[19-21,25,26] may oxidise GSH to GSSG (oxidazed glutathione) in circulating cells. Therefore, a reduction of GSH in the circulating cells of hypertensive patients may be expected. Plasma thiol and plasma glutathione are markedly lower in pregnancy-induced hypertension[57]. A decrease of GSH and an increase of GSSG have been observed in blood and in the peripheral mononuclear cells of hypertensive subjects when compared with those of age-matched controls. As a result, GSSG/GSH, a clear marker of oxidative stress in biological systems, is increased[33]. It must be pointed out that changes in the redox status of cells may have profound implications in terms of both metabolic as well as gene expression regulation[55-56]. In addition, reduced intracellular GSH levels below critical values, may lead to irreversible damage[58] and to a susceptibility to LDL oxidation. In gestational hypertension, a reduced GSH and an increased GSSG have been reported and the effect has been correlated with higher susceptibility of the erythrocytes of hypertensive pregnant women to oxidative stress challenge[59]. GSH depletion may be considered as an additional mechanism that may impact hypertension the level of oxidative stress. The depletion of GSH by the inhibition of GSH synthase causes severe hypertension in rats[60]. On the contrary, increasing intracellular GSH levels with N-acetylcysteine (NAC) has been shown to inhibit superoxide production by ANGII in the presence of NADH or of NADPH as substrates[61].

Oxidative stress byproducts in human hypertension

The number of oxidative markers discovered has been growing during the past two decades and still deserves research attention in order to find accurate metabolites for clinical applications. Methods for the quantitation of different types of oxidative stress indicators have been developed to reflect the oxidation products of the most important cell biomolecules including lipids, proteins, carbohydrates and nucleic acids[5] (table 2).

Lipids-based byproducts

Lipid peroxidation, the result of a ROS attack on membrane phospholipids is a general mechanism whereby oxidative stress induced tissue damage[62] has been classically determined by the yield of thiobarbituric acid reactive materials (TBARS)[63]. This method, however, has been criticized due to its poor specificity and to the majority of organic compounds being susceptible to ROS oxidation and producing substrates able to react with thiobarbituric acid[5]. Another byproduct, malodialdehide (MDA), has been shown to be increased in patients with cardiovascular diseases[64-66] and also recently in hypertensive subjects[33].

Isoprostanes

An isoprostane, 8-iso-prostaglandin $F_2\alpha$ has recently been proposed as an indicator of oxidative stress in smokers[44] as well as in patients with cardiovascular diseases[67-68]. Isoprostanes are free radical oxidation products of arachidonic acid. Two separate routes of peroxidation, an endoperoxide route and a dioxethane/endoperoxide mechanism, can form the isoprostanes. The isoprostanes in plasma have a short half life, approximately 18 minutes. They are rapidly excreted into urine suggesting that they must be constantly formed in order to maintain a steady-state concentration. In addition to their use as markers of oxidative stress, isoprostanes have potent biological activity in the vasculartur, where they interact with a new class of prostaglandin receptors exerting discrete effects on platelets and the endothelium[69].

Recently another possible marker of oxidative stress, isolevuglandin

adduct, has been described. Isolevuglandins are highly reactive gamma-keoaldehydes, which are formed via a non-enzymatic rearrangement in the isoprostane pathway. Isolevulgandin bind covalently with proteins, and cause protein-protein as well as protein-DNA cross-linking. The former are more closely correlated with cardiovascular disease than is the classical risk factor LDL of total cholesterol[70-71]. Other protein-bound products, as a result of MDA or 4-hydroxynonenal (4-HNE), have been also proposed as oxidative biomarkers[72].

Protein-based byproducts

The cysteine moiety of GSH is highly susceptible to oxidation by ROS. The free thiol group of cysteine readily undergoes reversible oxidation to form a disulphide, which can be reduced by the glutathione reductase system using GSH as a hydrogen donor. Glutathione oxidation to GSSG is a frequent event in oxidative stress. Thus GSSG/GSH ratio represents one of the most valuable markers of ROS-induced damage and cell redox status. Alterations of the GSSG/GSH status has been observed in erythrocytes of hypertensive pregnant women and compared with normotensive pregnant controls. In these cells, GSH stability decreases after an in vitro oxidative challenge, suggesting an impairment of the GSH recycling system and attributed to an insufficient NADPH supply[73]. More recently a reduction of GSH and an increase of GSSG and GSSG/GSH ratio has been reported in human hypertension[33].

Other protein-based biomarkers that have attracted interest for monitoring oxidative stress are isolated protein carbonyls, bityroisine, L-DOPA, and ortho-tyrosine[74]. No studies, however, have yet to be performed with these potential markers in HTN.

Deoxyribonucleic acid

Some of the most reliable markers of oxidative stress are the DNA damage bases, especially 8-oxo-dG which can be measured by different methods[74]. During the past few years the interest in 8-oxo-dG assay has significantly grown, based not only on its oxidative stress relation but also because of its mutagenic potential. The presence of 8-oxo-dG residues in DNA can lead to GC to TA transversion, unless repaired prior to DNA replication[75]. Therefore, the role of the oxidised form of guanine has been extensively studied and is still a matter of great interest in tumor research, while in other degenerative diseases, such as atherosclerosis or human hypertension the experimental data is sparse and requires further research[76]. Recently, in SHRSP rats, an increase of urine in the damaged base was observed only after severe hypertension[77]. In circulating blood cells, we were able to demonstrate a significant increase of 8-oxo-dG in hypertensive patients. In lymphocytes the damage base was also found significantly increased in mitochondrial DNA, suggesting that oxidative stress in hypertension is extensive and implicates different cell compartments. The impact of this effect on endothelial wall cells, however, is difficult to establish. Although a positive correlation was observed between the levels of 8-oxo-dG and MDA in hypertensive subjects, no relationship was observed between the average 24-hour mean blood pressure and the oxidative stress biomarkers analyzed[33].

Table 2 – Oxidative stress biomarkers

* Malondialdehyde (MDA)	Lipid peroxidation
* F_2-Isoprostanes	Lipid peroxidation
* Conjugates dienes/ethane	Lipid peroxidation
* Deoxyribose-induced MDA	Carbohydrates oxidation
* Amadori products	Carbohydrates oxidation
* Carbonyl groups	Protein oxidation
* Relación GSSG/GSH	Cellular redox state
* 8-oxo-dG	Oxidative DNA modification

· **Enzyme activities (SOD, catalase, GSH-peroxidase)**

· **Plasma antioxidant potential (TRAP-Assay)**

Assessment of oxidative stress in clinical studies

The importance of using appropriate methods to evaluate the magnitude of oxidative stress and to determine the correlation with hypertension and organ damage must be emphasized. All the studies previously outlined were performed in animal models or in tissue obtained from patients in experimental protocols. The growing importance of ROS raises the necessity of reproducible and reliable markers of oxidative stress, and that its assessment be repeateable over time so as to monitor treatment-induced changes. This leads to the measurement of ROS markers in blood or in

circulating cells, although correlation of ROS parameters between circulating and vascular wall cells has not been analyzed. Up to now it is not clear what type of cells from peripheral blood, if any, best reflect the oxidative stress present in the vascular wall.

Clinical significance of oxidative stress in hypertension

Assessment of antioxidant activities and lipid peroxidation byproducts in hypertensives indicates an excessive amount of ROS and a reduction of antioxidant mechanism activity in both blood as well as in several other cellular systems[78], including not only vascular wall cells[79], but also those cells found in circulating blood[80].

Our group has simultaneously examined the activities of the most important antioxidant enzymes, SOD, CAT and GPx, together with the levels of GSH, GSSG and the level ratios in blood and in the mononuclear peripheral cells from both hypertensive patients as well as from a subset of normotensive subjects[33]. Hypertensive patients exhibit important deficiencies of physiological antioxidants with an important reduction in the antioxidant mechanisms, both GSH levels as well as antioxidant enzymatic activities. Likewise, large quantities of peroxidation and DNA oxidation byproducts accumulate.

A decrease in the antioxidant enzymatic activities included all the enzymatic systems studied: SOD activity in plasma, mainly a Cu/Zn SOD3; SOD in peripheral mononuclear cells, mainly cytoplasmic Cu/Zn SOD1; and CAT and GPx in both plasma and peripheral mononuclear cells. Whether the low GSH levels and activity of the antioxidant enzymes are the cause or the consequence of the increased oxidative status needs further evaluation. The low activity included several systems which points to the reduction being more a consequence than a cause.

Reactive oxygen species oxidized GSH to GSSG leading to a decrease in the former and an increase in the latter. Moreover, even though the increment in ROS may upregulate the antioxidant enzymes under higher amounts of pure oxygen or related species, consumption by ROS can overcome the increased production leading to the low enzyme activity observed. Long time oxidative stress can consume antioxidants, and low activity levels of SOD, CAT and GPx have been reported in many other degenerative processes with an increase in ROS. An alternative explanation to low SOD, catalase and GPx activities is that these antioxidant enzymes are downregulated or inhibited by some factor in hypertensive individuals.

Among the ROS-induced byproducts, we observed an increase in MDA and in DNA oxidation. Malondialdehide, the most abundant among the reactive aldehydes derived from lipid peroxidation, was significantly increased in both blood as well as in peripheral mononuclear cells. These aldehydes have been implicated as causative agents in cytotoxic processes, and it is reasonable to suppose that releases from cell membranes may diffuse, interact and induce oxidative modifications in other cells and in LDL molecules, thereby increasing the risk of cardiovascular damage[81].

DNA oxidation in peripheral mononuclear cells from hypertensive subjects implies the involvement of the highly reactive hydroxyl radicals (-OH). These radicals readily react with guanosine to yield 8-oxo-dG, and it has been proposed as a good estimation of -OH formation. The contribution of other oxidants to DNA damage such as hypochlorite or peroxynitrite ions, however, also needs to be considered. Thus, in an environment in which H_2O_2 production or availability may be enhanced, as is the case of low catalase activity, reactions with chloride ions may lead to the formation of hypochlorite and singlet oxygen ($^1O_2$) through a myeloperoxidase-like reaction[82]. Moreover, peroxynitrite, derived from the combination of O_2^- with nitric oxide, is sufficiently reactive to induce DNA damage. Such damage influences the expression of several key stress-response genes in the regulation of cell cycle and cell proliferation[83]. Thus, the progressively increased DNA oxidation may induce an accelerated aging phenotype in the vascular cells of these patients.

The clinical impact of this enhanced ROS-activity is still not clear. The absence of a relationship between blood pressure values and oxidative stress in this group of hypertensive subjects may indicate that factors other than BP values alone. Factors inherent to the hypertensive status such as the enhanced activity of angiotensin II or hyperinsulinemia, may be responsible for the altered oxidative state in blood and in peripheral mononuclear cells. Even though no relationship between oxidative stress and BP values has been observed, such a relationship cannot be excluded by the data from the present study since blood pressure levels result from the interaction of a large number of regulatory systems capable of masking the association. An alternative explanation is that circulating ROS measurements do not adequately reflect oxidative stress in the vascular wall, since the enzymes which increase ROS have different

regulatory mechanisms in endothelial cells than they do in mononuclear peripheral cells.

Whether or not oxidative stress can contribute to the development of organ damage in hypertension remains a matter of debate[84]. An increase in ROS may reduce nitric oxide bioavailability contributing to the development of organ-damage[85]. Whatever the mechanisms implicated in ROS generation, an increase in oxidative stress has been associated with endothelial dysfunction[86] and seems to be related to HTN-induced organ dysfunction. Although several studies in humans have been focused on the relationship between ROS and endothelial function, as well as in the changes with antioxidant vitamins, the number of studies which focus in the organ-damage issue are scarce. Lacy et al[87], in a family-based cohort of hypertensives, observed that plasma hydrogen peroxide production was negatively correlated with cardiac contractility and renal function. Up to now there is not enough information to accept or refuse this hypothesis.

Finally, ROS changes by anti-hypertensive treatments have not been explored in depth. Several studies reported a reduction in the oxidative stress parameters during antihypertensive treatment although the mechanism has not been well researched.

Conclusions

Reactive oxygen species interfere with the mechanisms controlling BP and play an important role in the development of hypertension and vascular damage. Oxidative stress is increased in hypertensive subjects even in cells other than those present in the vascular wall. This increased oxidative stress, not related to BP values, is accompanied by a reduction in the most important antioxidant mechanisms and by the accumulation of ROS byproducts, not only from lipid peroxidation but also from oxidized genomic and mitochondrial DNA. The question of whether or not the reduction in antioxidant mechanisms is the cause or the consequence of oxidative stress needs to be explored in the near future to better understand the clinical significance of oxidative stress in hypertension.

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