JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2016, 67, 3, 331-337
www.jpp.krakow.pl

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ROLE OF GLUTATHIONE METABOLISM AND GLUTATHIONE-RELATED ANTIOXIDANT DEFENSE SYSTEMS IN HYPERTENSION

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The risk of developing chronic hypertension increases with age. Among others factors, increased oxidative stress is a well-recognized etiological factor for the development of hypertension. The co-occurrence of oxidative stress and hypertension may occur as a consequence of a decrease in antioxidant defense system activity or elevated reactive oxygen species generation. Glutathione is a major intracellular thiol-disulfide redox buffer that serves as a cofactor for many antioxidant enzymes. Glutathione-related parameters are altered in hypertension, suggesting that there is an association between the glutathione-related redox system and hypertension. In this review, we provide mechanistic explanations for how glutathione maintains blood pressure. More specifically, we discuss glutathione's role in combating oxidative stress and maintaining nitric oxide bioavailability *via* the formation of nitrosothiols and nitrosohemoglobin. Although impaired vasodilator responses are observed in S-nitrosothiol-deficient red blood cells, this potential hypertensive mechanism is currently overlooked in the literature. Here we fill in this gap by discussing the role of glutathione in nitric oxide metabolism and controlling blood pressure. We conclude that disturbances in glutathione metabolism might explain age-dependent increases in blood pressure.

Key words: *glutathione, glutathione peroxidase, glutathione reductase, glutathione transferase, hypertension, methemoglobin, nitrosoglutathione, oxidative stress*

INTRODUCTION

Hypertension is a known risk factor for morbidity and mortality from cardiovascular disease and affects as many as 70% of older adults. Age is an independent risk factor for both hypertension and cardiovascular disease (1, 2). The most common type of hypertension, essential (or primary) hypertension, accounts for 95% of cases. Hypertension is a complex, multifactorial disease involving multiple pathways and interactions between genetic and environmental factors (3). The etiology of hypertension has been attributed to a number of biological pathways including upregulation of the renin-angiotensin-aldosterone system, activation of the sympathetic nervous system, perturbed G protein-coupled receptor signaling, inflammation, and altered T-cell function and oxidative stress. The increased bioavailability of reactive oxygen species (ROS) (termed oxidative stress) due to excess ROS generation, decreased nitric oxide (NO) levels, and reduced antioxidant capacity in the cardiovascular, renal, and nervous systems are common features of these processes (4, 5).

ROS formation by oxidants and ROS elimination by antioxidants is usually finely balanced in vessel walls under physiological conditions. Elevated ROS formation in the vessel wall is a key feature of cardiovascular disease and contributes to endothelial dysfunction, vascular inflammation, and cardiovascular remodeling (6). Superoxide radicals ($O_2^{\bullet-}$) are known to have pro-hypertensive effects on vascular tone. Irrespective of their poor chemical reactivity, superoxide radicals are probably capable of interacting directly with specific intracellular targets as well as generating secondary cytotoxic species such as peroxynitrite ($ONOO^-$) (7), which is formed via rapid radical-radical coupling reactions between $O_2^{\bullet-}$ and nitric oxide (NO) in the vascular wall. Furthermore, enhanced inactivation of the vasodilator NO by $O_2^{\bullet-}$ may explain the observed relationship between ROS, in particular superoxide overproduction, and high blood pressure. In line with the above argument it has been shown that the mechanism of restoration of endothelial function is related to favorably increased the [NO]/[($ONOO^-$)] balance, enhanced endothelial cytoprotective NO, decreased cytotoxic ($ONOO^-$) (8).

Regardless of the evidence for a possible role for reactive species in endothelial dysfunction and the pathophysiology of essential hypertension, the relationship between blood pressure and redox biology is not fully elucidated. Further studies are

required to identify free radicals and their intermediates, evaluate evidence of radical attack on molecules, and estimate antioxidant status in hypertension. In our previous clinical studies, we hypothesized that the cellular glutathione antioxidant defense system plays an important role in maintaining blood pressure in hypertensive patients. For instance, we observed significant disturbances in glutathione and glutathione-related enzyme levels in elderly patients treated for hypertension (9). Moreover, glutathione plays an important role in NO metabolism: glutathione reacts with peroxynitrite from S-nitrosothiols (RSNOs), which subsequently release NO over a prolonged time to extend the half-life of NO, thereby preventing the negative effects of NO scavenging by superoxide. This role of glutathione in nitric oxide availability shows that this antioxidant may have a particular function in maintaining blood pressure in excess of its antioxidant actions. For instance Partridge *et al.* (10) examined gene expression in vascular smooth muscle cells to study the complex interaction between oxidative injury and the pathogenesis of vascular disease. Extensive vascular remodeling was coupled to altered increased production of F2-isoprostane, 8-epi-prostaglandin F2 alpha, nuclear localization of NFkappaB and altered glutathione homeostasis.

In this review we discuss the role of the glutathione system in hypertension. First, we provide a general introduction to glutathione metabolism in relation to its antioxidant role and oxidative stress. Next, we review the evidence for an association between the glutathione system and hypertension, with a focus on reduced glutathione, glutathione peroxidase, and glutathione transferase. Finally we provide some specific mechanistic explanations for the role that glutathione plays in NO metabolism, and in doing so also discuss S-nitrosohemoglobin-mediated vasodilation and the role of glutathione system in the control of blood pressure with increasing age.

OXIDATIVE STRESS AND GLUTATHIONE

Many human physiological processes generate unstable and highly reactive chemical molecules known as reactive oxygen species (ROS) and reactive nitrogen species (RNS). These moieties are formed by the univalent reduction of oxygen in mitochondria, where the superoxide radical ($O_2^{\bullet-}$) is formed, followed by hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) formation. Other reactive species with either oxidizing or nitrating properties include the nitric oxide radical ($NO^{\bullet}$), peroxynitrite (ONOO-), and the lipid peroxyl radical ($LOO^{\bullet}$). When production of reactive molecules with oxidizing properties overwhelms the cellular antioxidant capacity, oxidative stress occurs, resulting in molecular and cellular tissue damage and severe metabolic malfunction and pathological sequelae such as hypertension. There is considerable experimental evidence showing that increased pro-oxidant production and decreased antioxidant bioavailability play an important role in the pathophysiology of hypertension and endothelial injury (11). To prevent radical-mediated toxicity, specific enzymes and low molecular weight substances such as glutathione (GSH) eliminate reactive species and help maintain cellular redox balance.

Reduced glutathione (GSH) is a tripeptide comprised of glutamic acid, cysteine, and glycine that is synthesized *de novo* from its constituent amino acids *via* two adenosine triphosphate (ATP)-dependent enzymatic reactions. GSH synthesis is catalyzed by glutamate cysteine ligase (GCL, previously known as GCS) and GSH synthetase. Transcription and activity of γ-GCS is regulated by many factors and processes including GSH depletion, GSH conjugation, antioxidant levels, inflammatory cytokines, nitrosative stress, and oxidative stress (12). GSH is synthesized in the cytosol of all mammalian cells at a rate dependent on the availability of cysteine (its sulfur amino acid precursor) and the activity of the rate-limiting enzyme GCL, altered activity of which is associated with altered cellular GSH in many conditions. GCL gene expression is upregulated under conditions where Zn increased cellular defense response is necessary; for instance, oxidative stress is known to induce the expression of GSH synthesis enzymes, GCL activity, and GSH levels. Key transcription factors regulating the expression and induction of GCLC by pro- and anti-oxidants include nuclear respiratory factor NRF2/NRF1, activator protein-1 (AP-1), and nuclear factor kappa B (NFκB). Recently, c-Myc has also been shown to contribute to the basal expression and induction of human GCL under conditions of oxidative stress.

Glutathione serves as a major intracellular thiol-disulfide redox buffer. GSH has an easily oxidizable sulfhydryl group that protects against oxidant injury by both enzymatic and non-enzymatic mechanisms. GSH is readily oxidized to its disulfide form (GSSG), which can be transformed back to its GSH form, by reduced nicotinamide adenine dinucleotide phosphate (NADPH) and the FAD-dependent glutathione reductase (GR). Most glutathione is maintained as GSH in healthy humans and animals, with GSSG concentrations generally being low. Pro-oxidant overproduction is accompanied by activation of the glutathione defense system and a significant increase in reduced glutathione levels and glutathione reductase activity to protect proteins and membrane lipids from oxidation. Several glutathione-dependent enzymatic antioxidant pathways protect the cell from oxidant injury: glutathione peroxidase (GPx) uses GSH as a cofactor to reduce H_2O_2 and organic hydroperoxides, the overall reaction reducing peroxide molecules to water and oxidatively coupling two glutathiones to liberate GSSG (13). Glutathione transferase (GST) forms a second phase of detoxification, catalyzing deactivation of a number of harmful compounds, requiring reduced glutathione as a cofactor, which is irreversibly consumed during GSH conjugation. Detoxification of xenobiotics and/or their metabolites is another major function of GSH (14).

Since many metabolic pathways result in a decrease in GSH and an increase in GSSG concentrations, the restoration of reduced glutathione is crucial for maintaining cellular homeostasis. Glutathione reductase (GR), which catalyzes the reaction $NADPH + GSSG + H^+ \rightarrow NADP^+ + 2GSH$, plays an important role in the maintenance of cellular oxidant status (15). However, severe oxidative stress can overcome the capacity of the cell to reduce GSSG to GSH, leading to GSSG accumulation. The cell is protected from large shifts in redox equilibrium by active extracellular transport of GSSG or reaction with protein sulfhydryl groups and the formation of mixed disulfides. Protracted oxidative stress and diminished antioxidant defenses are associated with GSH oxidation and depletion (16), a phenomenon observed in hypertensive subjects compared to non-hypertensive subjects (17).

HYPERTENSION AND GLUTATHIONE-RELATED PARAMETERS OF OXIDATIVE STRESS

Hypertension is associated with disturbances in glutathione metabolism. Decreased levels of red blood cell reduced glutathione (RBC-GSH) and increased levels of oxidized glutathione have been observed in hypertensive patients, resulting in an elevated ratio of oxidized to reduced glutathione compared to controls. Furthermore, in hypertensive patients (but not controls), RBC-GSH is inversely correlated with systolic blood pressure (18).

In another study, significantly higher ratios of oxidized to reduced glutathione and significantly lower activities of glutathione peroxidase were detected in whole blood and peripheral mononuclear cells in hypertensive patients compared to normotensive volunteers re, a finding supported by another study reporting significantly lower GSH and higher GSSG values in mononuclear cells from hypertensive subjects compared to controls (19). Furthermore, the activity of γ-GCS, GR, and GPx glutathione metabolism enzymes has also been shown to be dysregulated in hypertension. The decreased activity of antioxidant enzymes in hypertension has been attributed to impaired expression and enzyme inactivation during oxidative stress. The inadequate response of the main cytoplasmic antioxidant systems and the enzymes participating in glutathione maintenance may contribute to the vulnerability of hypertensive patients to oxidative stress. Conversely, it has been shown that antihypertensive treatment reduces oxidative stress and results in increased GSH, decreased GSSG, and significant enhancement of enzymes involved in glutathione metabolism (19). The latter is consistent with our own findings of increased GSH concentrations in treated hypertensive patients (9).

Given this strong evidence suggesting an association between glutathione metabolism and hypertension, it is important to seek a mechanistic explanation for how glutathione may play a role in the pathophysiology of hypertension.

OXIDATIVE STRESS AND BLOOD PRESSURE CONTROL - THE ROLE OF GLUTATHIONE IN THE NITRIC OXIDE METABOLISM

Blood pressure (BP) control can be regarded as the product of the control of blood flow to a given tissue in proportion to its metabolic need. Local mechanisms controlling blood flow include acute and chronic vasoconstriction and dilatation and changes in the tissue vasculature, with endothelial autocrine secretions playing an important role in vasoconstriction and vasodilation. In addition to the local control of blood flow, the autonomic nervous system mediates global control of blood flow including changes in cardiac output and control of arterial BP: while the sympathetic nervous system (SNS) controls arterial hypertension, the parasympathetic nervous system contributes primarily to the regulation of cardiac function. Coordination between arterial homeostasis and the renin-angiotensin-aldosterone system (RAAS), the endocrine system regulating blood pressure and water (fluid) balance, is the most powerful BP control mechanism (20).

The RAAS is considered to be a major activator of NADPH oxidase and ROS production in hypertension. Angiotensin II stimulates NADPH oxidase (NOX) by: (i) increasing expression of NADPH oxidase subunits; and (ii) increasing ROS production in vascular smooth muscle cells, endothelial cells, and fibroblasts. Several NADPH oxidases may play a role in blood pressure control (21), and ROS from NADPH oxidases can also trigger adaptive signaling that improves glutathione replenishment *via* redox-dependent increases in glutathione reductase activity. The positive role of glutathione in preventing hypertension may also be explained by the observations that high glutathione levels prevent platelet-derived growth factor (PDGF)-mediated production of ROS by NADH/NADPH oxidase, which is considered to be the main source of vascular ROS (7), (22). Therefore, glutathione/glutathione disulfide concentrations and NADPH oxidase activity maintain the redox state and control mitochondrial ROS production (23). In the case of excessive ROS production, other redox-sensitive systems are affected, such as expression of endothelial nitric oxide synthase (eNOS) (4). eNOS is the predominant NOS isoform in vessels and is responsible for most of the vascular NO production. NO generated by eNOS regulates vascular tone by inhibiting smooth muscle contraction and platelet aggregation. NO can further regulate vascular smooth muscle tone with ROS by introducing reversible S-nitroso- and S-glutathione adducts on cysteine thiols.

Liu *et al.* demonstrated that S-nitrosothiols play an essential role in NO biology and influence blood pressure (24). S-nitrosothiols formed from the NO-dependent S-nitrosation of thiol-containing proteins and peptides such as albumin and glutathione are implicated in the *in vivo* transport, storage, and metabolism of NO. These data suggest that increased GSH levels should have a positive effect on BP in hypertension, and we propose that pathological inhibition of vascular relaxation involves not only NO production by endothelial cells but also glutathione-dependent bioavailability of NO, with S-nitrosothiols playing an important role in the pathway. Similar mechanisms may be responsible for the enhanced vasodilatory action of peroxynitrite (ONOO) in the presence of GSH: peroxynitrite reacts with GSH to form S-nitrosothiols (RSNOs), which subsequently release NO over a prolonged period of time, thus extending NO's half-life and, as a result, relaxing vascular tone (25). The main source of peroxynitrite is from inactivation of NO by superoxide, the best-known vasoconstricting ROS.

The vasodilatory action of peroxynitrite is enhanced in the presence of GSH, an effect attributed to a guanylate cyclase-dependent mechanism induced by the reaction between GSH and peroxynitrite to form the NO donor S-nitrosoglutathione (GSNO). NO activates soluble guanylate cyclase and is reported to regulate and stimulate coronary vascular tone (26). GSNO is an efficient NO donor in the presence of GSH and Cu/Zn superoxide dismutase (CuZn-SOD). SOD-dependent catalysis of NO formation from RSNOs occurs in the presence of GSH, which reduces the active site copper in SOD to the cuprous oxidation state, which in turn rapidly reduces GSNOs to NO (27). For instance, we have shown that elderly patients with treated hypertension exhibit increased CuZn-SOD activity and elevated GSH levels (28), which may suggest that lowering blood pressure during antihypertensive treatment is at least in part due to a CuZn-SOD-GSH-dependent mechanism. Liu *et al.* demonstrated that S-nitrosothiols are essential in NO biology and influence blood pressure (24), and it has also been reported that thiol supplementation with GSH selectively improves human endothelial dysfunction by improving NO bioavailability (29).

In summary, GSNO can be regarded as protective against oxidative stress and a stabilizer and carrier of NO in hypertension (30). Nitrosothiols also have a beneficial effect on vascular tone due to their ability to inhibit NADPH oxidase (31). However, increased GSNO levels have been observed in inflammatory states and subsequent induction of inducible nitric oxide synthase (iNOS), which is also related to hypertension (32). The links between hypertension and S-nitrosoglutathione are clearly complex and suggest that, depending on the milieu created by different biochemical processes, GSNO is either a friend or foe of the cardiovascular system. A schematic of the mechanisms controlling blood pressure is depicted in *Fig. 1*.

GLUTATHIONE PEROXIDASE PLAYS A CONTRADICTORY ROLE IN HYPERTENSION

With respect to hypertension, glutathione peroxidase (GPx) has important functions in the reduction of peroxides that are reported to inactivate vasodilating NO and the decomposition of S-nitrosoglutathione (GSNO), which plays an important role in vascular homeostasis (33). GPx is particularly vulnerable to the oxidative stress associated with hypertension. For instance, it has been shown that $O_2^{\bullet -}$, an important hypertensive radical, can inhibit this peroxidase (34). GPx also appears to have an opposing

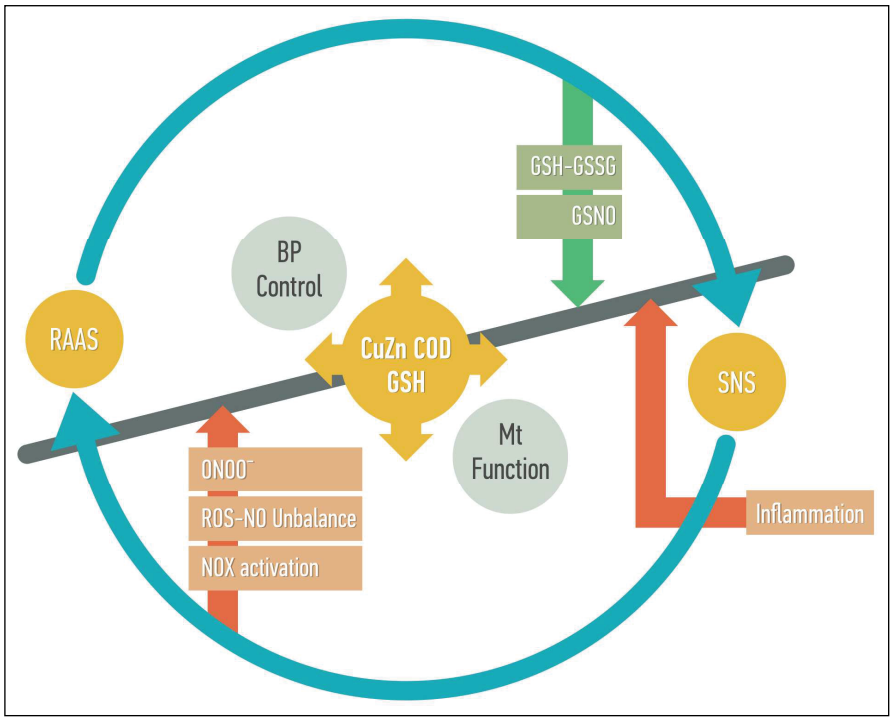

Fig. 1. Mechanisms involved in blood pressure control. When the renin-angiotensin-aldosterone system, the sympathetic nervous system, and inflammatory processes lead to NADPH oxidase activation, the mitochondrial dysfunction, and NO-ROS imbalance, a proper cell concentrations of glutathione/glutathione disulfide maintain the redox state and NO availability. *Abbreviations:* BP, blood pressure; Cu/Zn SOD, copper-zinc superoxide dismutase; GSH-GSSG, reduced glutathione-glutathione disulfide; GSNO, S-nitrosoglutathione; Mt, mitochondria; NO, nitric oxide; $ONOO^-$, peroxynitrite; NOX, NADPH oxidase; RAAS, renin-angiotensin-aldosterone system; ROS, reactive oxygen species; SNS, sympathetic nervous system.

role in free radical metabolism. Although a number of studies have pointed to a protective role for GPx in overcoming the oxidative injury and cell death mediated by ROS *in vivo*, it has also been reported to potentiate reactive nitrogen species (RNS). In this respect, GPx seems to be a double-edged sword, playing opposite roles in regulating ROS and RNS ((35) and see (36) for a review of the opposing roles of GPx in oxidative stress). Some specific examples of this phenomenon include GPx-1 preventing hepatocyte apoptosis caused by ROS but potentiating hepatocyte apoptosis caused by RNS. *In vivo*, obesity and insulin resistance in mice were associated with GPx-1 overexpression, further supporting a dual role for GPx-1 in regulating different types of oxidative stress with a possible deleterious impact on metabolism, molecular, and physiological functions. With respect to GPx-1 in hypertension, our own study was the first clinical evidence (9) to shed light on the notion that the relationship between the level of antioxidants including GPx-1 and clinical pathologies is not always straightforward and that the protective role of increased activities of antioxidant enzymes depends on other factors including the characteristics of the oxidative milieu. Nevertheless, most studies have mainly focused on the protective role of GPx in hypertension. For instance, the seleno-glutathione peroxidase mimic ebselen (PZ51) protected the endothelium and vasculature of stroke-prone spontaneously-hypertensive rats (SHRsp) during chronic hypertension (37), indicating an important role for this enzyme in the maintenance of vascular homeostasis (37). Further research is needed to elucidate the role of GPx-1 in hypertension in relation to the overall metabolic and oxidant status and the specific interrelationship between GPx-1 and relevant biological parameters.

RELATIONSHIP BETWEEN GLUTATHIONE TRANSFERASE AND HYPERTENSION

GST plays an important in role in the detoxification of ROS and limiting oxidative damage in tissues by catalyzing conjugation of GSH with various electrophiles, physiological metabolites, and xenobiotics. An increase in GST activity suggests increased GSH demand for conjugation reactions, where conjugation itself regulates GSH synthesis by increasing γ-GCS transcription (12).

GST represents a family of isoenzymes with several different classes (alpha, kappa, mu, omega, pi, theta) located in the cytosol, mitochondria, and microsomes. GST theta (encoded by *GSTT1*) and mu (encoded by *GSTM1*) have been examined in hypertension. *GSTT1* has 2 alleles denoted *GSTT1*0* for the non-functional allele and *GSTT1*1* for the functional allele (38). *GSTT1*-positive carriers display higher GST activity and may exhibit relative protection against oxidative stress and oxidative DNA damage (39). However, the clinical data on the association between *GSTT1* and hypertension is inconclusive. Nevertheless, the *GSTM1*0/GSTT1*0* genotype has been shown to be a potential genetic risk factor for essential hypertension (40), and it has been reported that genetic loss of the GSTT1 enzyme is an independent and powerful predictor of premature vascular morbidity and death in individuals with type 2 diabetes (41).

It has been also shown that disruption in *Gstm1* in mice is conducive to methemoglobinemia (42). Indeed, it has been suggested that GSTs may function as heme binding and transport proteins (43). Given these data, we postulate that GST helps to overcome methemoglobinemia in hypertensive patients, the mechanism of which is discussed below.

S-NITROSOHEMOGLOBIN-MEDIATED VASODILATION

We have already discussed some of the interactions between nitrosothiols and blood pressure, but here we explain how hemoglobin also participates in these processes. S-nitrosoglutathione (GSNO) is, as already mentioned, an NO donor and, in red blood cells, this mechanism involves hemoglobin. GSNO participates in the formation of the Cysβ93-nitrosated derivative of hemoglobin, S-nitrosohemoglobin (SNO-Hb), which has been suggested to play significant physiological role by acting as an endogenous NO donor and thus a regulator of blood pressure. The important role of glutathione in NO bioavailability in red blood cells is further

supported by evidence suggesting that the presence of low molecular weight thiols (e.g., glutathione) is a prerequisite for the release of NO from SNO-Hb (44). SNO-Hb was originally thought to be an oxygen-sensitive controller of vascular tone such that, on deoxygenation, the nitroso moiety was transferred to glutathione to form GSNO (30, 45). In fact, at GSH concentrations within the physiological range in red blood cells, SNO-Hb can still promote vasodilation even under oxygenated conditions (46). More recently, it has been suggested that NO from GSNO may be converted to SNO-Hb prior to its release from RBCs (47), perhaps due to the ability of S-nitrosothiols to react with ferrous heme groups in oxyHb; thus, oxyheme inhibits GSNO-mediated relaxation (46). There is also evidence to suggest that the vasoconstrictive effect related to scavenging of NO by the oxygenated heme can be reversed in the presence of glutathione, especially under anaerobic conditions (30).

We propose that the role of SNO-Hb-GSH system in controlling blood pressure increases with age due to changes in erythrocyte metabolism. More specifically, this change may be related to increased Hb oxidation and, as a consequence, metHb production. With increasing concentrations of metHb, Hb loses the ability to bind and release O_2. Under such conditions, mechanisms responsible for hypoxic vasodilation are activated such as the GSH-dependent mechanism of NO release from SNO-Hb (29).

MetHb is unable to bind and carry oxygen. A small percentage of Hb is always auto-oxidized under normal physiological conditions resulting in the presence of metHb in blood, but metHb can also be present due to genetic abnormalities, exposure of red cells to certain drugs or toxins, or due to a deficit in antioxidants. To avoid metHb accumulation, reducing pathways mediated by cytochrome b5 or flavin coupled with NADH- or NADPH-dependent metHb reductases (MHbR), respectively, keep the level of metHb in erythrocytes at less than 1% of the total Hb under normal conditions (48).

However, metHb is a reversible phenomenon of the oxidant-antioxidant balance and under conditions of increased oxidative stress and decreased MHbR activity, it can accumulate more readily. It has been reported that age is associated with decreased MHbR activity (49). Moreover, age is associated with increased oxidative stress (50). To protect cells from the overproduction of pro-oxidants and methemoglobinemia, glutathione-related antioxidant defense systems are activated (51).

In as much as MHbR activity attenuates with age, increasing levels of metHb limit the amount of free nitrite, which is thought to be the largest intravascular pool of vasoactive NO. A possible role for changes in hemoglobin-oxygen affinity in the pathogenesis of hypertension has been already considered (52). Other negative effects of metHb accumulation include its toxicity related to protein tyrosine nitration, protein oxidation, and lipid oxidation (53). Moreover, in the presence of H_2O_2, metHb can perform a peroxidative reaction (i.e., it exhibits pseudoperoxidase activity), resulting in the formation of oxoferrylHb, a strong oxidant with the iron in the ferryl state Fe(IV) (54). Studies on the peroxidase activity of the ferrylhemoglobin (ferrylHb) radical have shown that $O_2^{\bullet -}$ is produced when it is reduced to metHb (55). Considering increasing levels of metHb and redox imbalance with age, intraerythrocytic H_2O_2 peroxidation giving rise to an important source of vasoconstricting superoxide radicals may, as a consequence, cause hypertension. The effective suppression of metHb formation seems to be an important mechanism for maintaining health over time and successful aging (56). Consistent with the latter, the suppressive effect of GSH on metHb formation should not be disregarded (57). However, under normal conditions, this pathway is likely to be less significant, only becoming important when MHbR activity is disrupted (58).

GLUTATHIONE AND AGE-RELATED PATHOPHYSIOLOGY OF HYPERTENSION

Since aging is associated with oxidative stress and the glutathione redox system is affected by age (59), decreased GSH might be a risk factor for the development of age-related toxicity and diseases including hypertension.

Epidemiological studies have shown that advancing age is associated with an increased prevalence of hypertension (60), and genetic and physiological effects on blood pressure are modulated by age (61, 62). Since advancing age is a risk factor for developing hypertension, we must consider that age-related disturbances in glutathione metabolism might be contributory (63, 64). Decreased (65), increased (66), and unaltered (67) glutathione levels with age have been reported. Results related to GPx activity have also been contradictory, and whether GPx activity decreases (67) or increases with age remains inconclusive. Age-related disturbances in glutathione reductase activity have been also reported (59), and an increasing frequency of glutathione transferase GSTT1 homozygous deletions is also associated with age (7, 22, 68-70).

CONCLUSIONS

Together, this evidence suggests that oxidative stress and in particular disturbed glutathione metabolism are related to the pathogenesis of hypertension. Glutathione-related antioxidant defense systems appear to be altered in hypertensive patients, leading to increased oxidative stress, reduced NO bioavailability, and, as a consequence, elevated blood pressure. We have provided a comprehensive analysis of the evidence suggesting that redox balance plays an important role in controlling blood pressure. The mechanistic role of glutathione in NO bioavailability suggests that this moiety might control blood pressure, in particular under conditions of oxidative stress. Recognizing and better understanding the role of glutathione in blood pressure control also provides an opportunity to develop new therapeutic strategies. Both basic and clinical studies have provided evidence that glutathione deficiency not only participates in pathology, but its replacement offers an opportunity for powerful therapeutic intervention. To date, the administration of glutathione precursors has been successfully used to treat hepato- and neurotoxicity; therefore, more evidence is needed to assess the potential of such therapies in vascular toxicity including hypertension. However, the failure of many antioxidant clinical studies suggests that a sound mechanistic approach is ultimately needed to improve the design of future studies.

Acknowledegments: This study was supported by a grant to Joanna Rybka (currently Robaczewska) 'Stipends for doctorate students - 2008/2009 - ZPORR' financed by European Union from the European Social Fund and The Integrated Regional Operational Programme (IROP).

Abbreviations: BP, blood pressure; Cu/Zn-SOD, Cu/Zn superoxide dismutase; eNOS, endothelial nitric oxide synthase; GCL, glutamate cysteine ligase; GR, glutathione reductase; GSH, glutathione; GSNO, S-nitrosoglutathione; GSSG, glutathione disulfide; GST, glutathione transferase; H_2O_2, hydrogen peroxide; NADPH, nicotinamide adenine dinucleotide phosphate; $NO^{\bullet}$, nitric oxide radical; NOS, nitric oxide synthase; NOX, NADPH oxidase; $O_2^{\bullet}$, superoxide radical; $OH^{\bullet}$, hydroxyl radical; $ONOO^-$,

peroxynitrite; RAAS, renin-angiotensin-aldosterone system; ROS, reactive oxygen species; RNS, reactive nitrogen species; RSNOs, S-nitrosothiols; SNO-Hb, S-nitrosohemoglobin

Conflicts of interests: None declared.

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Received: September 4, 2012
Accepted: June 24, 2016

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