Cardiovascular and Renal Manifestations of Glutathione Depletion Induced by Buthionine Sulfoximine

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Oxidative stress contributes to the development of several cardiovascular diseases, including diabetes, renal insufficiency, and arterial hypertension. Animal studies have evidenced the association between higher blood pressure (BP) and increased oxidative stress, and treatment with antioxidants has been shown to reduce BP, while BP reduction due to antihypertensive drugs is associated with reduced oxidative stress. In 2000, it was first reported that oxidative stress and arterial hypertension were produced in normal Sprague-Dawley rats by oral administration of buthionine sulfoximine (BSO), which induces glutathione (GSH) depletion, indicating that oxidative stress may induce hypertension. The contribution of several potential pathogenic factors has been evaluated in the BSO rat model, the prototype of oxidative stress-induced hypertension, including vascular reactivity, endothelium-derived factors, renin–angiotensin system activity, TXA_2-PGH_2 production, sodium sensitivity, renal dopamine-induced natriuresis, and sympathetic tone. This review summarizes the main factors implicated in the pathogenesis of BSO-induced hypertension and the alterations associated with GSH depletion that are related to renal function or BP control.

Keywords: *blood pressure; buthionine sulfoximine (BSO); glutathione; hypertension; oxidative stress*

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Animal studies have provided evidence that increased blood pressure (BP) is associated with increased oxidative stress, which has been demonstrated in spontaneous (genetic)[1,2] and experimental models of hypertension, induced by angiotensin II,[3] deoxycorticosterone acetate-salt,[4] lead,[5] T[4,6] and NO synthase inhibition.[7] However, findings on the association between oxidative stress and hypertension in humans have been less consistent, and results have varied according to the oxidative stress marker under investigation.[8]

In animal hypertension models, many authors reported that antioxidation may reduce BP,[1–7] while others found no such association.[9,10] In human studies, antihypertensive drug therapy with angiotensin II type 1 (AT_1) blockers[11] or calcium antagonists[12] has proven to have beneficial effects on oxidative stress.

It was first reported in 2000[13] that oxidative stress and arterial hypertension were produced in normal Sprague-Dawley rats by oral administration of buthionine sulfoximine (BSO), which induces glutathione (GSH) depletion by selective inhibition of γ-glutamylcysteine synthetase, an enzyme of the GSH biosynthetic pathway. This model of hypertension is attenuated by antioxidant therapy[13] and aggravated by increased saline intake,[14] by administration of diethyldithiocarbamic acid (DETC), an inhibitor of superoxide dismutase, and by the hyperthyroid state (data not shown), which are all known to increase oxidative stress.

Grossman[15] proposed the following criteria for the identification of oxidative stress as a cause of hypertension: (i) oxidative stress should be associated with hypertension, (ii) the mechanism by which oxidative stress causes hypertension should be known, (iii) oxidative stress should cause hypertension in experimental animals, and (iv) antioxidation should lower the BP. According to the available evidence, BSO-induced hypertension is a prototype of hypertension caused by increased oxidative stress. This review describes the cardiovascular and renal effects of BSO-induced oxidative stress (**Figure 1**).

BLOOD PRESSURE

Discrepant BP results have been reported after BSO-induced GSH depletion. Authors indirectly measuring BP by tail cuff measurements have variously reported a marked increase (around 100 mm Hg),[13,16] a modest increase (20 mm Hg),[17,18] and even no change.[19] Conflicting results have also been published when the BP was directly recorded. Ford *et al.*[20] found that BSO (20 or 30 mmol/l in drinking water) had no significant effect on direct systolic BP levels measured via indwelling catheters in two locations (femoral and carotid) on repeated occasions over 10 days in awake, unrestrained animals or at the end of this period under anesthesia. In contrast, several studies by Banday *et al.*[14,21,22] reported a modest increase (13–23 mm

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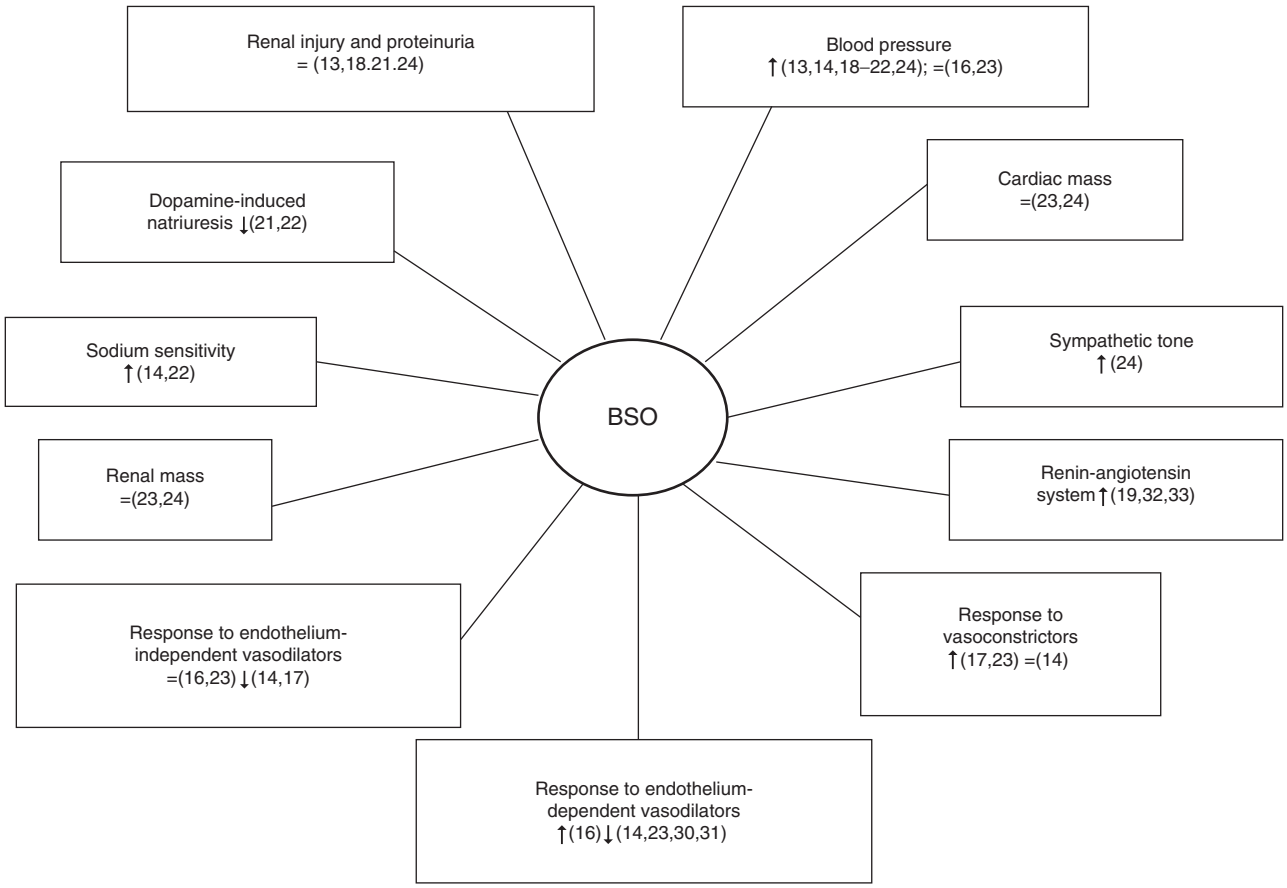

Figure 1 | Summary of the effects of increased oxidative stress induced by buthionine sulfoximine (BSO) administration to rats or mice on cardiovascular and renal variables involved in blood pressure control or hypertension. ↑ increased, = unchanged and ↓decreased.

Hg) in anesthetized rats. All of these discrepancies are difficult to reconcile, but cannot credibly be attributed to differences in BP recording method or rat strain (Sprague-Dawley in all cases) or to a central effect of BSO that predisposes these animals to excessive BP increases under stress, as previously suggested by Ford *et al.*[20] In mice, BSO was reported to induce a dose-dependent BP increase, with the highest dose used (20 mmol/l in drinking water) producing an increase of around 50 mm Hg.[23] Hypertensive stimuli usually produce a lesser BP increase in mice than in rats.[24] Thus, after 4 weeks of hypertension induction, systolic BP was found to be around 200 mm Hg in renovascular or deoxycorticosterone acetate-salt hypertensive rats and around 140 mm Hg in mice.[25]

HEART RATE

BSO administration was found to produce a dose-related increase in heart rate (HR) in mice.[23] Results of the few studies on this variable in rat hypertension models have been inconclusive. Ford *et al.*[20] found no significant effect of BSO on HR across sampling days or when measured via indwelling catheter in the carotid artery of anaesthetized rats after 10 days of treatment. However, Ganafa *et al.*[26] administered BSO to Sprague-Dawley rats for 24 h and observed a parallel time course for mean arterial pressure (MAP) and HR values, although they found no HR modification after BSO treatment for 1 week.[26]

An increase in oxidative stress induced by the systemic administration of the superoxide dismutase inhibitor DETC was found to produce a rise in MAP, HR, and renal sympathetic nerve activity (RSNA)[27] that was reversed by the systemic administration of tempol, an antioxidant superoxide dismutase mimetic.[25,27,28] These observations suggest that the dose–response increase in HR reported in BSO-treated mice may be secondary to an increased sympathetic activity induced by greater oxidative stress.

CARDIAC AND RENAL HYPERTROPHY

Relative left ventricular weight and renal weight relative to body weight were unchanged in BSO-treated mice, despite

their hypertension.[23] These observations are similar to the findings of Ford *et al.*[20] in rats treated with BSO for 10 days at 20 and 30 mmol/l and those of Johns *et al.*,[24] who unexpectedly observed that renovascular and deoxycorticosterone acetate-salt hypertensive mice did not develop cardiomegaly after 4 weeks of hypertension induction, in contrast to the widely reported cardiac hypertrophy of these models in rats. All of these findings suggest a reduced sensitivity to develop cardiomegaly in mice. Our group also observed (data not shown) that mice treated with 10 mmol/l BSO had a significantly decreased expression of α-tubulin in renal tissue at 55 kDa and showed another band of protein at 40 kDa, which may correspond to a carbonylated form of the protein. Cytoskeletal proteins are particularly susceptible to oxidative stress, and the formation of protein carbonyls during GSH depletion has been demonstrated in rat brain slices, identifying β-actin and α/β-tubulin as major carbonylation targets.[29] These findings may in part explain the absence of cardiac and renal hypertrophy and even the weight loss observed in BSO-treated mice, given that an adequate level of cytoskeletal proteins is crucial for tissue development.

VASCULAR REACTIVITY

BSO-induced GSH depletion causes vasomotor dysfunction in the setting of oxidative stress. However, contradictory results have been reported for its effects on the responsiveness to vasoconstrictors or endothelium-dependent or -independent vasodilators.

Ganafa *et al.*[26] found an increased BP responsiveness to phenylephrine at 24 h after BSO administration. Likewise, Ford *et al.*[20] reported that a 10-day course of BSO produced an increased sensitivity and maximal vasoconstrictor response of aortic rings to phenylephrine, but they found no significant differences in KCl dose–response curves between BSO and control groups. However, Banday *et al.*[14] found no difference in the maximum contraction produced by phenylephrine or KCl in aortic rings of rats treated with BSO for 12 days.

An *in vitro* study of acute GSH depletion demonstrated reduced acetylcholine (ACh)- and NO-induced relaxation of rabbit aortic rings.[30] In an *in vivo* study by Laursen *et al.*, BSO (intraperitoneal for 24 h) reduced the hypotensive effect of ACh by 30%, and the impaired effect of ACh was associated with a significant reduction in endothelial nitric oxide synthase (eNOS) activity.[31] The authors concluded that thiol depletion results in endothelial dysfunction and a reduced receptor-mediated vascular relaxation due to diminished endothelial NO formation. An *in vitro* study by Ford *et al.*[20] also reported that BSO significantly blunted endothelium-dependent relaxation to ACh in aortic rings of rat. Likewise, Banday *et al.*[14] showed a mild rightward shift to ACh in thoracic aortic rings from rats treated with BSO for 12 days and found that concomitant treatment of rats with BSO and high salt produced a decreased response to endothelium-dependent agonists (ACh, ADP, and calcium ionophore A23187). In contrast, Iwata *et al.*[19] found a significantly enhanced ACh-induced vasodilation in aortic rings from BSO-treated rats (30 mmol/l in drinking water for 7 days) in comparison to control rats. This was thought to be due to eNOS-dependent H_2O_2 production, because ACh-induced relaxation was significantly reduced by catalase and completely abolished by L-NAME. The authors reported a more abundant eNOS expression in BSO-treated vs. control rats and suggested that the uncoupling of eNOS may contribute to the production of O_2^-, which is dismuted to H_2O_2 as the result of SOD activity, thereby enhancing vasorelaxation. The contradictory nature of these data prevents a definitive conclusion from being drawn on endothelial function after BSO administration.

Ganafa *et al.*[26] described an impaired response to the endothelium-independent sodium nitroprusside at 24 h after BSO administration. Banday *et al.*[14] also found a decreased response to endothelium-independent vasorelaxants in thoracic aortic rings from rats treated with BSO for 12 days, and showed a decreased response to endothelium-independent vasorelaxants in rats treated concomitantly with BSO and high salt; moreover, incubation of aortic tissue from BSO-treated rats with sodium nitroprusside revealed decreased cyclic guanosine monophosphate accumulation. In contrast, Ford *et al.*[20] and Iwata *et al.*[19] showed a similar endothelium-independent vasorelaxation to sodium nitroprusside between aortas from control and BSO-treated rats, demonstrating that responsiveness of the vascular smooth muscle to NO was not affected by the treatment. Taken together, the above findings suggest that BSO-induced GSH depletion results in oxidative stress-dependent vasomotor dysfunction, which might participate in the reported hypertensive effect of BSO. However, further research is required to clarify this issue.

VASOACTIVE AGENTS

Various groups reported a significant reduction in the plasma or urinary excretion of the NO metabolite nitrate plus nitrite, an index of depressed NO availability, in BSO-treated rats.[13,16,26] BSO-treated animals showed no significant differences in the intensity or localization of eNOS or inducible nitric oxide synthase in the kidney,[16] whereas increased eNOS levels were observed in the aorta.[14,19] These observations suggest that the oxidative stress-induced hypertension in this model is not caused by depressed NOS expression by the vessels or the kidney or by their depressed NOS expression, but is rather associated with and perhaps partially related to enhanced renal NO inactivation by reactive oxygen species (ROS) and diminished NO bioavailability.

Ganafa *et al.*[26] reported that the acute administration of BSO reduced plasma prostacyclin at 8 and 24 h, whereas NO was reduced at 24 h, when thromboxane A_2 was increased in

both plasma and aorta. The chronic administration of BSO (1 or 2 weeks) by these authors increased isoprostane and thromboxane A_2 but decreased prostacyclin and cAMP,[17,18] indicating that BSO administration reduces vasodilator and increased vasoconstrictor agents.

Rats treated chronically with BSO (BSO, 30 mmol/l in drinking water for 2 weeks) showed increased plasma angiotensin II levels, whereas administration of the AT_1 blocker losartan reversed the BSO-induced elevation of MAP, superoxide, and thromboxane A_2 and reduction of prostacyclin and aortic cAMP levels.[17] BSO treatment also increased renal proximal tubular AT_1 receptor protein abundance, message levels, and ligand binding.[32] More recently, these authors[33] observed that the incubation of renal proximal tubules with angiotensin II produced significantly higher NHE3 activation in BSO-treated rats than in controls, indicating that oxidative stress exaggerates angiotensin II signaling, leading to the overstimulation of renal NHE3 and contributing to an increase in BP. All of the above data suggest an important role for the renin–angiotensin system in this type of hypertension.

Sympathetic tone

Sympathetic tone plays an important role in BP control,[34] and a growing body of evidence has implicated abnormal modulation of the sympathetic nervous system (SNS) in the development of hypertension in humans[35] and animals.[36] SNS activity was augmented in salt-loaded spontaneously hypertensive rats and Dahl salt-sensitive rats,[36–38] while α_1-adrenergic blockade with prazosin produced a marked BP decrease in NO-deficient hypertensive rats.[39]

It has been reported that ROS stimulate the central and peripheral SNS.[40,41] Central neural ROS play an important role in cardiovascular function, and the altered regulation of central redox mechanisms may be implicated in heart failure and hypertension, which are characterized by SNS activation.[42,43] Among the target organs of hypertensive vascular diseases, the brain is the most affected by oxidative stress,[44,45] and there is evidence that oxidative stress in the brainstem has a pivotal role in the pathogenesis of neural mechanisms in hypertension.[46] The rostral ventrolateral medulla is the vasomotor center that determines basal sympathetic nerve activity.[47] ROS are increased in the rostral ventrolateral medulla of stroke-prone spontaneously hypertensive rats and contribute to the neural mechanisms of this type of hypertension,[48] and oxidative stress was detected in the rostral ventrolateral medulla and paraventricular nucleus of the hypothalamus in renovascular hypertension.[49,50]

Several authors have reported that the hypotensive effect of tempol, a membrane-permeable superoxide dismutase mimetic, is accompanied by a reduction in sympathetic nerve activity.[25,27,28,43,51] In addition, the intracerebroventricular infusion in rabbits of DETC, a superoxide dismutase inhibitor, significantly increased RSNA,[52] and the systemic administration of DETC in rats increased the MAP, HR, and RSNA.[16] Moreover, the local administration of tempol or DETC on renal sympathetic nerves resulted in dose-dependent decreases or increases in integrated RSNA, respectively.[27] More recently, it was reported that chronic vitamin C treatment of two-kidney one-clip hypertensive rats reduced arterial pressure and RSNA and improved cardiac and renal baroreflex responses.[53]

Several studies have reported that oxidative stress increases sympathetic tone in some types of experimental hypertension, including genetic,[48] angiotensin II,[43] deoxycorticosterone acetate-salt,[51] renovascular,[50] obesity,[54] and salt-sensitive[55] models. High-salt intake exacerbates BP elevation and SNS system activity during the development of hypertension in spontaneously hypertensive rats, mediated by increased ROS generation in the rostral ventrolateral medulla.[56] Moreover, the protective central actions of estrogen in angiotensin II-induced hypertension may involve interactions with ROS production.[57] In addition, the BP-lowering effect of some antihypertensive drugs has been associated with a reduction in brainstem oxidative stress.[58–60] Taken together, these observations suggest an important role for oxidative stress-induced sympathetic activation in hypertension. This proposition is supported by findings[23] that BSO-induced hypertension in mice is accompanied by increased brainstem and urinary levels of isoprostanes and increased plasma levels of noradrenaline, that chronic α_1-adrenergic blockade with prazosin prevents the hypertension and increased HR of BSO-treated mice, and that acute ganglionic blockade with pentolinium produces greater percentage MAP and HR decreases in BSO-treated mice than in controls. Accordingly, it was proposed that BSO increases central and peripheral oxidative stress, which may produce a central or peripheral activation of sympathetic tone that elevates resting BP and HR in BSO-treated mice.

Increased oxidative stress was previously shown to be an important mechanism leading to impairment of the reflex control of circulation in experimental hypertension.[53] However, no data are available on the actions of BSO-induced oxidative stress in central or peripheral nervous system control of the cardiovascular system by arterial baroreceptors.

The precise mechanism by which oxidative stress may raise the sympathetic tone in BSO-treated animals has not been fully elucidated. Local ROS production appears to mediate SNS activation in the brain, NO modulates SNS activity in the central nervous system, and increased ROS production can activate the SNS through the oxidation/inactivation of NO.[61] In Sprague-Dawley rats, superoxide dismutase mimetics increased the abundance of neuronal nitric oxide synthase in brain nuclei involved in the noradrenergic control of BP.[62] As reported above, BSO-treated rats show reduced plasma and

urinary levels of the NO metabolite nitrate plus nitrite.[13,16,26] These observations suggest that the increased SNS activity produced by BSO administration may be related to a decreased NO bioavailability due to ROS-induced NO inactivation.

RENAL FUNCTION AND DOPAMINE-INDUCED NATRIURESIS

Serum creatinine concentration, creatinine clearance, and urinary protein excretion values were similar between BSO-treated animals and controls.[13,16,21,23] Furthermore, light microscopy studies found no morphological abnormalities in the kidneys of BSO-treated rats[13,16,21] or mice.[23] These observations suggest that oxidative stress per se cannot induce discernible renal disease and/or structural abnormalities of the kidney, at least in the period analyzed in these studies, i.e., < 5 weeks. However, it has been reported that BSO pretreatment may exacerbate renal mercury toxicity in rats.[63]

Dopamine plays an important role in regulating renal function and BP. Dopamine synthesis and dopamine receptor subtypes have been demonstrated in the kidney. Dopamine acts via cell surface receptors coupled to G proteins linked to adenylyl cyclase stimulation. Activation of D_1-like receptors on the proximal tubules inhibits tubular sodium reabsorption by inhibiting Na/H-exchanger and Na/K-adenosine triphosphatase activity. There have been reports of defective renal dopamine production and/or dopamine receptor function in human primary hypertension and in genetic models of animal hypertension.[64,65]

BSO treatment (30 mmol/l for 2 weeks) caused oxidative stress and an increase in BP, which was accompanied by defective D_1 receptor G-protein coupling and loss of natriuretic response to SKF38393, a D_1 receptor agonist. BSO treatment also increased NF-κβ nuclear translocation, protein kinase C activity and expression, G-protein–coupled receptor kinase-2 membranous translocation, and D_1 receptor serine phosphorylation.[21] In BSO-treated rats, supplementation with tempol decreased oxidative stress, normalized BP, and restored D_1 receptor G-protein coupling and natriuretic response to SKF38393. Subsequently, this group[22] also observed that the concomitant administration of BSO (30 mmol/l for 12 days) and high salt caused oxidative stress, D_1 receptor dysfunction, and a marked increase in BP. Although renal dopamine production was increased, the baseline Na/K-ATPase activity was not reduced in these animals. Treatment of rats with BSO and high salt plus the antioxidant tempol decreased oxidative stress, restored endogenous and exogenous D_1 receptor agonist-mediated Na/K-ATPase inhibition, and normalized BP.

CONCLUDING REMARKS

Over the past two decades, a large number of experimental studies have reported the functions and roles of free radicals in normal physiology and have described the association of oxidative stress with diverse disease states, including arterial hypertension. The finding that oral administration of BSO, that induces GSH depletion, produced oxidative stress and increased BP, demonstrated for the first time that oxidative stress can be a cause of hypertension and offered the opportunity to study the interaction of oxidative stress with factors that elevate BP. This review summarizes the main factors implicated in the pathogenesis of BSO-induced hypertension and the alterations related to renal function and BP control (**Figure 1**), offering a thorough and updated analysis of the contribution of several potential pathogenic factors, including vascular reactivity, renin–angiotensin system activity, TXA2-PGH2 production, sodium sensitivity, renal dopamine-induced natriuresis, and sympathetic tone.

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1. Schnackenberg CG, Wilcox CS. Two-week administration of tempol attenuates both hypertension and renal excretion of 8-Iso prostaglandin f2alpha. *Hypertension* 1999; 33:424–428.
2. Duarte J, Pérez-Palencia R, Vargas F, Ocete MA, Pérez-Vizcaino F, Zarzuelo A, Tamargo J. Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. *Br J Pharmacol* 2001; 133:117–124.
3. Kopkan L, Castillo A, Navar LG, Majid DS. Enhanced superoxide generation modulates renal function in ANG II-induced hypertensive rats. *Am J Physiol Renal Physiol* 2006; 290:F80–F86.
4. Galisteo M, García-Saura MF, Jiménez R, Villar IC, Zarzuelo A, Vargas F, Duarte J. Effects of chronic quercetin treatment on antioxidant defence system and oxidative status of deoxycorticosterone acetate-salt-hypertensive rats. *Mol Cell Biochem* 2004; 259:91–99.
5. Vaziri ND, Ding Y, Ni Z, Gonick HC. Altered nitric oxide metabolism and increased oxygen free radical activity in lead-induced hypertension: effect of lazaroid therapy. *Kidney Int* 1997; 52:1042–1046.
6. Moreno JM, Rodríguez Gómez I, Wangensteen R, Osuna A, Bueno P, Vargas F. Cardiac and renal antioxidant enzymes and effects of tempol in hyperthyroid rats. *Am J Physiol Endocrinol Metab* 2005; 289:E776–E783.
7. Sainz J, Wangensteen R, Rodríguez Gómez I, Moreno JM, Chamorro V, Osuna A, Bueno P, Vargas F. Antioxidant enzymes and effects of tempol on the development of hypertension induced by nitric oxide inhibition. *Am J Hypertens* 2005; 18:871–877.
8. Ward NC, Croft KD. Hypertension and oxidative stress. *Clin Exp Pharmacol Physiol* 2006; 33:872–876.
9. Zhang GX, Kimura S, Nishiyama A, Shokoji T, Rahman M, Abe Y. ROS during the acute phase of Ang II hypertension participates in cardiovascular MAPK activation but not vasoconstriction. *Hypertension* 2004; 43:117–124.
10. Elmarakby AA, Loomis ED, Pollock JS, Pollock DM. NADPH oxidase inhibition attenuates oxidative stress but not hypertension produced by chronic ET-1. *Hypertension* 2005; 45:283–287.
11. Chamorro V, Wangensteen R, Sainz J, Duarte J, O'Valle F, Osuna A, Vargas F. Protective effects of the angiotensin II type 1 (AT1) receptor blockade in low-renin deoxycorticosterone acetate (DOCA)-treated spontaneously hypertensive rats. *Clin Sci* 2004; 106:251–259.
12. Taddei S, Virdis A, Ghiadoni L, Versari D, Salvetti G, Magagna A, Salvetti A. Calcium antagonist treatment by lercanidipine prevents hyperpolarization in essential hypertension. *Hypertension* 2003; 41:950–955.

13. Vaziri ND, Wang XQ, Oveisi F, Rad B. Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. *Hypertension* 2000; 36: 142–146.
14. Banday AA, Muhammad AB, Fazili FR, Lokhandwala M. Mechanisms of oxidative stress-induced increase in salt sensitivity and development of hypertension in Sprague-Dawley rats. *Hypertension* 2007; 49:664–671.
15. Grossman E. Does increased oxidative stress cause hypertension? *Diabetes Care* 2008; 31 Suppl 2:S185–S189.
16. Bayorh MA, Ganafa AA, Socci RR, Eatman D, Silvestrov N, Abukhalaf IK. Effect of losartan on oxidative stress-induced hypertension in Sprague-Dawley rats. *Am J Hypertens* 2003; 16:387–392.
17. Bayorh MA, Ganafa AA, Socci RR, Eatman D, Silvestrov N, Abukhalaf IK. Effect of losartan on oxidative stress-induced hypertension in Sprague-Dawley rats. *Am J Hypertens* 2003; 16:387–392.
18. Ganafa AA, Walton M, Eatman D, Abukhalaf IK, Bayorh MA. Amlodipine attenuates oxidative stress-induced hypertension. *Am J Hypertens* 2004; 17:743–748.
19. Iwata C, Wang X, Uchida K, Nakanishi N, Hattori Y. Buthionine sulfoximine causes endothelium dependent hyper-relaxation and hypoadiponectinemia. *Life Sci* 2007; 80:873–878.
20. Ford RJ, Graham DA, Denniss SG, Quadrilatero J, Rush JW. Glutathione depletion ***in vivo*** enhances contraction and attenuates endothelium-dependent relaxation of isolated rat aorta. *Free Radic Biol Med* 2006; 40:670–678.
21. Banday AA, Fazili FR, Lokhandwala MF. Oxidative stress causes renal dopamine D1 receptor dysfunction and hypertension via mechanisms that involve nuclear factor-kappaB and protein kinase C. *J Am Soc Nephrol* 2007; 18:1446–1457.
22. Banday AA, Lau YS, Lokhandwala MF. Oxidative stress causes renal dopamine D1 receptor dysfunction and salt-sensitive hypertension in Sprague-Dawley rats. *Hypertension* 2008; 51:367–375.
23. Rodríguez-Gómez I, Baca Y, Moreno JM, Wangensteen R, Perez-Abud R, Payá JA, O'Valle F, Vargas F. Role of sympathetic tone in BSO-induced hypertension in mice. *Am J Hypertens* 2010; 23:882–888.
24. Johns C, Gavras I, Handy DE, Salomao A, Gavras H. Models of experimental hypertension in mice. *Hypertension* 1996; 28:1064–1069.
25. Raij L, Dalmasso AP, Staley NA, Fish AJ. Renal injury in DOCA-salt hypertensive C5-sufficient and C5-deficient mice. *Kidney Int* 1989; 36:582–592.
26. Ganafa AA, Socci RR, Eatman D, Silvestrova N, Abukhalaf IK, Bayorh MA. Acute inhibition of glutathione biosynthesis alters endothelial function and blood pressure in rats. *Eur J Pharmacol* 2002; 454:217–223.
27. Shokoji T, Fujisawa Y, Kimura S, Rahman M, Kiyomoto H, Matsubara K, Moriwaki K, Aki Y, Miyatake A, Kohno M, Abe Y, Nishiyama A. Effects of local administrations of tempol and diethyldithio-carbamic on peripheral nerve activity. *Hypertension* 2004; 44:236–243.
28. Chen X, Patel K, Connors SG, Mendonca M, Welch WJ, Wilcox CS. Acute antihypertensive action of Tempol in the spontaneously hypertensive rat. *Am J Physiol Heart Circ Physiol* 2007; 293:H3246–H3253.
29. Bizzozero OA, Reyes S, Ziegler J, Smerjac S. Lipid peroxidation scavengers prevent the carbonylation of cytoskeletal brain proteins induced by glutathione depletion. *Neurochem Res* 2007; 32:2114–2122.
30. Adachi T, Cohen RA. Decreased aortic glutathione levels may contribute to impaired nitric oxide-induced relaxation in hypercholesterolaemia. *Br J Pharmacol* 2000; 129:1014–1020.
31. Laursen JB, Boesgaard S, Trautner S, Rubin I, Poulsen HE, Aldershvile J. Endothelium-dependent vasorelaxation in inhibited by *in vivo* depletion of vascular thiol levels: role of endothelial nitric oxide synthase. *Free Radic Res* 2001; 35:387–394.
32. Banday AA, Lokhandwala MF. Oxidative stress-induced renal angiotensin AT1 receptor upregulation causes increased stimulation of sodium transporters and hypertension. *Am J Physiol Renal Physiol* 2008; 295:F698–F706.
33. Banday AA, Lokhandwala MF. Oxidative stress causes renal angiotensin II type 1 receptor upregulation, Na+/H+ exchanger 3 overstimulation, and hypertension. *Hypertension* 2011; 57:452–459.
34. DiBona GF. Sympathetic nervous system and the kidney in hypertension. *Curr Opin Nephrol Hypertens* 2002;11:197–200.
35. Fujita T, Henry WL, Bartter FC, Lake CR, Delea CS. Factors influencing blood pressure in salt-sensitive patients with hypertension. *Am J Med* 1980; 69:334–344.
36. Koepke JP, DiBona GF. High sodium intake enhances renal nerve and antinatriuretic responses to stress in spontaneously hypertensive rats. *Hypertension* 1985; 7:357–363.
37. Ono A, Kuwaki T, Kumada M, Fujita T. Differential central modulation of the baroreflex by salt loading in normotensive and spontaneously hypertensive rats. *Hypertension* 1997; 29:808–814.
38. Leenen FH, Ruzicka M, Huang BS. The brain and salt-sensitive hypertension. *Curr Hypertens Rep* 2002; 4:129–135.
39. Wangensteen R, O'Valle F, Del Moral R, Vargas F, Osuna A. Chronic alpha1-adrenergic blockade improves hypertension and renal injury in L-NAME and low- renin L-NAME-DOCA hypertensive rats. *Med Sci Monit* 2002; 8: BR378–BR384.
40. Campese VM, Ye S, Zhong H, Yanamadala V, Ye Z, Chiu J. Reactive oxygen species stimulate central and peripheral sympathetic nervous system activity. *Am J Physiol Heart Circ Physiol* 2004; 287:H695–H703.
41. Zanzinger J, Czachurski J. Chronic oxidative stress in the RVLM modulates sympathetic control of circulation in pigs. *Pflugers Arch* 2000; 439:489–494.
42. Lindley TE, Doobay MF, Sharma RV, Davisson RL. Superoxide is involved in the central nervous system activation and sympathoexcitation of myocardial infarction-induced heart failure. *Circ Res* 2004; 94:402–409.
43. Zimmerman MC, Lazartigues E, Lang JA, Sinnayah P, Ahmad IM, Spitz DR, Davisson RL. Superoxide mediates the actions of angiotensin II in the central nervous system. *Circ Res* 2002; 91:1038–1045.
44. Ohtsuki T, Matsumoto M, Suzuki K, Taniguchi N, Kamada T. Mitochondrial lipid peroxidation and superoxide dismutase in rat hypertensive target organs. *Am J Physiol* 1995; 268:H1418–H1421.
45. Kimoto-Kinoshita S, Nishida S, Tomura TT. Age-related change of antioxidant capacities in the cerebral cortex and hippocampus of stroke-prone spontaneously hypertensive rats. *Neurosci Lett* 1999; 273:41–44.
46. Hirooka Y. Role of reactive oxygen species in brainstem in neural mechanisms of hypertension. *Auton Neurosci* 2008; 142:20–24.
47. Dampney RA. Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev* 1994; 74:323–364.
48. Kishi T, Hirooka Y, Kimura Y, Ito K, Shimokawa H, Takeshita A. Increased reactive oxygen species in rostral ventrolateral medulla contribute to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats. *Circulation* 2004; 109:2357–2362.
49. Oliveira-Sales EB, Dugaich AP, Carillo BA, Abreu NP, Boim MA, Martins PJ, D'Almeida V, Dolnikoff MS, Bergamaschi CT, Campos RR. Oxidative stress contributes to renovascular hypertension. *Am J Hypertens* 2008; 21:98–104.
50. Oliveira-Sales EB, Nishi EE, Carillo BA, Boim MA, Dolnikoff MS, Bergamaschi CT, Campos RR. Oxidative stress in the sympathetic premotor neurons contributes to sympathetic activation in renovascular hypertension. *Am J Hypertens* 2009; 22:484–492.
51. Xu H, Fink GD, Galligan JJ. Nitric oxide-independent effects of tempol on sympathetic nerve activity and blood pressure in DOCA-salt rats. *Am J Physiol Heart Circ Physiol* 2002; 283:H885–H892.
52. Gao L, Wang W, Li YL, Schultz HD, Liu D, Cornish KG, Zucker IH. Superoxide mediates sympathoexcitation in heart failure: roles of angiotensin II and NAD(P)H oxidase. *Circ Res* 2004; 95:937–944.
53. Nishi EE, Oliveira-Sales EB, Bergamaschi CT, Oliveira TG, Boim MA, Campos RR. Chronic antioxidant treatment improves arterial renovascular hypertension and oxidative stress markers in the kidney in Wistar rats. *Am J Hypertens* 2010; 23: 473–480.
54. Nagae A, Fujita M, Kawarazaki H, Matsui H, Ando K, Fujita T. Sympathoexcitation by oxidative stress in the brain mediates arterial pressure elevation in obesity-induced hypertension. *Circulation* 2009; 119:978–986.
55. Fujita M, Ando K, Nagae A, Fujita T. Sympathoexcitation by oxidative stress in the brain mediates arterial pressure elevation in salt-sensitive hypertension. *Hypertension* 2007; 50:360–367.
56. Koga Y, Hirooka Y, Araki S, Nozoe M, Kishi T, Sunagawa K. High salt intake enhances blood pressure increase during development of hypertension via oxidative stress in rostral ventrolateral medulla of spontaneously hypertensive rats. *Hypertens Res* 2008; 31:2075–2083.
57. Xue B, Zhao Y, Johnson AK, Hay M. Central estrogen inhibition of angiotensin II-induced hypertension in male mice and the role of reactive oxygen species. *Am J Physiol Heart Circ Physiol* 2008; 295:H1025–H1032.
58. Kishi T, Hirooka Y, Mukai Y, Shimokawa H, Takeshita A. Atorvastatin causes depressor and sympatho-inhibitory effects with upregulation of nitric oxide synthases in stroke-prone spontaneously hypertensive rats. *J Hypertens* 2003; 21:379–386.

59. Araki S, Hirooka Y, Kishi T, Yasukawa K, Utsumi H, Sunagawa K. Olmesartan reduces oxidative stress in the brain of stroke-prone spontaneously hypertensive rats assessed by an ***in vivo*** ESR method. *Hypertens Res* 2009; 32:1091–1096.
60. Chan SH, Wu CA, Wu KL, Ho YH, Chang AY, Chan JY. Transcriptional upregulation of mitochondrial uncoupling protein 2 protects against oxidative stress-associated neurogenic hypertension. *Circ Res* 2009; 105: 886–896.
61. Campese VM. Oxidative stress and sympathetic activity in hypertension. *Am J Hypertens* 2010; 23:456.
62. Ye S, Zhong H, Yanamadala S, Campese VM. Oxidative stress mediates the stimulation of sympathetic nerve activity in the phenol renal injury model of hypertension. *Hypertension* 2006; 48:309–315.
63. Xu Z, Yang J, Yu J, Yin Z, Sun W, Li J. Effects of BSO, GSH, Vit-C and DMPS on the nephrotoxicity of mercury. *Toxicol Ind Health* 2007; 23:403–410.
64. Banday AA, Lokhandwala MF. Dopamine receptors and hypertension. *Curr Hypertens Rep* 2008; 10:268–275.
65. Banday AA, Lokhandwala MF. Defective renal dopamine D1 receptor function contributes to hyperinsulinemia-mediated hypertension. *Clin Exp Hypertens* 2006; 28:695–705.