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News & Views

Lisinopril as an Antioxidant in Hypertension?

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ABSTRACT

Lisinopril is an inhibitor of the renin–angiotensin system. Does lisinopril minimize oxidative damage in spontaneously hypertensive rats (SHR) in comparison with the normotensive genetic controls Wistar Kyoto (WKY)? The authors note that lisinopril contained lipid peroxidation, elevated tissue glutathione levels, and influenced the activity of antioxidant enzymes such as catalase and glutathione peroxidase. Studies in humans testing the hypothesis that lisinopril has antioxidant function *in vivo* are warranted. *Antioxid. Redox Signal.* 9, 393–397.

OXIDATIVE STRESS AND HYPERTENSION

OXIDATIVE REACTIONS, which provide high-energy compounds in aerobic organisms, are a source of potentially cytotoxic reactive oxygen species (ROS) that induce tissue dysfunction through various pathways and contribute to numerous pathological conditions such as hypertension, sclerosis inflammation, heart failure, and other cardiovascular disorders (23). Studies performed in animal models have documented the existence of ROS overproduction in hypertension (21, 27).

Oxidative stress may contribute to nitric oxide (NO) inactivation (24, 25, 28), as well as to the generation of vasoconstrictive isoprostranes (1), leading to cellular damage evidenced by lipid peroxidation and changes in the antioxidant defenses (3).

Different antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (Gpx), and catalase (CAT) protect cells from deleterious effects of primary free radical species and participate in tissue detoxification. Hypertension also correlates with ROS-mediated alterations in the vasculature and myocardium, or in the central nervous system where downregulation of endogenous antioxidants has been observed (14).

We have previously demonstrated renal oxidative stress in spontaneously hypertensive rats (SHR) in which the activity of the antioxidant enzyme CAT was increased while Gpx and SOD were decreased in comparison with the normotensive group of Wistar Kyoto (WKY) rats (22). In agreement with these results, it has been reported that a different hypertensive model (nontreated SHR with uninephrectomy) showed decreased Cu/Zn SOD expression (15).

Vascular oxidative stress has also been demonstrated in experimentally induced hypertension, such as angiotensin (Ang) II-mediated hypertension, Dahl salt-sensitive hypertension, obesity-induced hypertension, and aldosterone-provoked hypertension (14).

THE RENIN–ANGIOTENSIN SYSTEM STIMULATES THE PRODUCTION OF REACTIVE OXYGEN SPECIES IN HYPERTENSION

The accumulated experimental evidence indicates that the renin–angiotensin system (RAS) plays a crucial role in the production of oxygen radicals in oxidative tissue injury (1). Ang II exerts classic hemodynamic and renal effects, but it is also a local biologically active mediator with direct effects on endothelial and smooth muscle cells and plays important roles in the development of cardiovascular diseases, including hypertension, cardiac hypertrophy, congestive heart failure, ischemic heart disease, and renal diseases (16). Ang II is thought to increase ROS production by activating vascular NAD(P)H oxidases (12).

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DO THE ANGIOTENSIN II TYPE 1 RECEPTOR BLOCKERS IMPROVE THE ANTIOXIDANT STATUS IN HYPERTENSION?

Many physiological functions of Ang II are directly mediated by ROS (13). It induces oxidative stress in heart, brain, and liver of the hypertensive rat model by decreasing the activity of antioxidant enzymes, while it plays no role in this process in normotensive genetic controls (22). When we examined the capacity of losartan, the Ang type 1 receptor blocker, to modify parameters of oxidative stress in SHR and normotensive control rats, changes were only detected in the SHR group (22).

For this reason, the aim of the present study was to determine whether the RAS inhibitor, lisinopril, is able to diminish the oxidative damage induced in liver, kidney, and brain from SHR in comparison with the normotensive WKY rats.

ANGIOTENSIN-CONVERTING ENZYME INHIBITORS DIMINISH ANG II PRODUCTION AND INCREASE OTHER BENEFICIAL ANTIHYPERTENSIVE METABOLITES

The angiotensin-converting enzyme (ACE) inhibitors are frequently used for clinical therapeutics of hypertension and cardiac hypertrophy. ACE inhibition diminishes Ang II production and, simultaneously, prolongs bradykinin half-life by inhibition of kinase II and activation of the kallikrein–kinin system (18). Furthermore, ACE inhibitors increase levels of the antihypertensive heptapeptide Ang-(1–7), that opposes various Ang II effects (29).

ANTIOXIDANT DEFENSES IN EXPERIMENTAL HYPERTENSION

In agreement with earlier results (22), the comparison of parameters of oxidative stress in tissues from hypertensive and normotensive rats indicated that no significant difference was observed in thiobarbituric acid reactive substances (TBARS) levels measured in liver, kidney, or brain homogenates (Fig. 1). In contrast, the glutathione (GSH) content varied among tissues, being lower in liver, but higher in brain from SHR (Fig. 2). Accordingly, the activity of antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx)] decreased in SHR tissues when compared to the WKY rats (Table 1). In SHR kidney, only CAT activity increased 25% with respect to normotensive rats (Table 1)

Therefore, it is clear that hypertension is associated with impaired antioxidant defenses in the SHR model.

DOES LISINOPRIL PLAY A ROLE AS ANTIOXIDANT IN HYPERTENSION?

In several pathologies, TBARS formation is a reliable indicator of free radical production in tissues. Present results show that lisinopril administration to SHR was associated with a diminished TBARS content in liver (35%), kidney (25%), and brain (40%). In addition, an increased GSH content was determined in liver (66%), kidney (31%), and brain (20%), in comparison with untreated SHR. Also, when the ACE inhibitor was given to SHR, changes in the activity of antioxidant enzymes were observed (Table 1). SOD, CAT, and Gpx activities increased in kidney 91%, 20%, and 30% and in brain 70%, 34%, and 30%, respectively, relative to values detected in untreated SHR. Surprisingly, in liver, the only lisinopril-promoted change was a 36% decreased CAT activity.

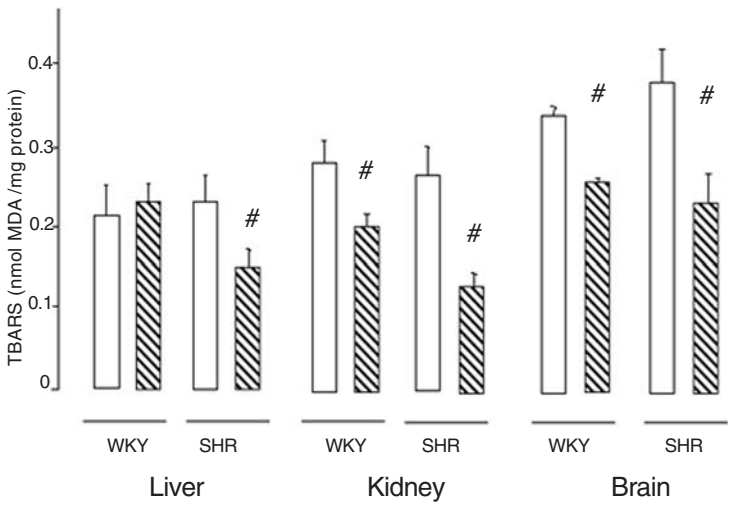

FIG. 1. Lipid peroxidation in tissues of control and lisinopril-treated rats. *Bars* represent mean ± SD of 8 animals in the WKY group and 8 animals in the SHR group. #$p < 0.0001$ lisinopril-treated group (*striped bars)* versus untreated control (*plain bars*).

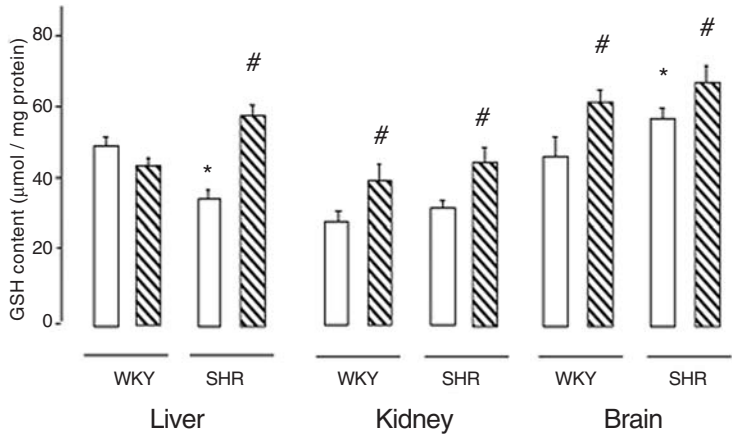

FIG. 2. Comparison of glutathione content in tissues of control and lisinopril-treated rats. *Bars* represent mean ± SD of 8 animals in the WKY group and 8 animals in the SHR group. #$p < 0.001$ lisinopril-treated group (*striped bars*) versus untreated control (*plain bars*). *$p < 0.001$ SHR versus WKY rats.

DOES LISINOPRIL EXERT SIMILAR ANTIOXIDANT EFFECTS IN THE NORMOTENSIVE WISTAR KYOTO GROUP?

After lisinopril administration, a significantly reduced lipid peroxidation was observed in kidney (30%) and brain (25%) of WKY rats, compared with the corresponding tissue of the untreated-WKY group (Fig. 1). Moreover, such treatment increased 27% the GSH level in kidney and 41% in brain (Fig. 2)

The ACE inhibitor also produced changes in the activity of antioxidant enzymes in WKY rats (Table 1), that is, it enhanced SOD and Gpx activities in kidney (30% and 19%, respectively) and brain (21% and 50%, respectively), relative to the untreated-WKY group.

On the contrary, lisinopril administration to normotensive controls decreased the activity of CAT (19%) and Gpx (11%) in liver (Table 1). This different pattern of response to lisinopril in liver, also observed in SHR, may be due to changes of ACE activity and/or different levels of expression or regulation of the RAS cascade in this organ. Besides, the pattern of ACE induction and inhibition might be different depending on the organ. Furthermore, it is necessary to consider that the effect of ACE inhibitors on the antioxidant defenses might occur only in certain tissues or cell types (4).

As support of our findings, it was reported that the administration of ACE inhibitors enalapril or captopril to normotensive animals does induce antioxidant enzymes in different tissues (4).

CONCLUSION AND OPEN QUESTIONS

Changes in the redox state of blood vessels and essential organs such as heart and kidney impair important functions leading to pathogenesis of hypertension. Kidneys are important blood pressure regulators that are also likely targets of ROS leading to elevated levels of oxidation markers and decreased urinary nitrate and nitrite, whereas the associated tissue damage results in protein excretion (14).

In the present study, lisinopril administration to SHR reduced lipid peroxidation, activated the antioxidant enzymes, and enhanced glutathione content, the most important nonenzymatic intracellular ROS scavenger in kidney and brain. These findings suggest that lisinopril may play an active role restoring glutathione content and/or stimulating cellular antioxidant mechanisms.

Similarly, ACE inhibition in a rat model of streptozotocin-induced diabetes reduced oxidative stress to the same extent

TABLE 1. ACTIVITY OF ANTIOXIDANT ENZYMES IN LIVER, KIDNEY, AND BRAIN OF NORMOTENSIVE AND HYPERTENSIVE RATS

		WKY	*WKY-Lis*	*SHR*	*SHR-Lis*
Liver	SOD (U/mg protein)	10.60 ± 2.56	8.02 ± 1.29**	3.22 ± 1.00$^{\&}$	4.00 ± 0.90
	CAT (pmol/mg protein)	3.10 ± 0.22	2.52 ± 0.24*	2.50 ± 0.29$^{\&}$	1.60 ± 0.10*
	Gpx (U/ mg protein)	0.55 ± 0.01	0.49 ± 0.01*	0.32 ± 0.01$^{\&}$	0.35 ± 0.03
Kidney	SOD (U/mg protein)	5.40 ± 0.70	7.02 ± 0.65*	2.80 ± 0.50$^{\&}$	5.35 ± 0.46*
	CAT (pmol/mg protein)	1.46 ± 0.38	1.41 ± 0.08	1.82 ± 0.10$^{\#}$	2.18 ± 0.10*
	Gpx (U/ mg protein)	0.59 ± 0.02	0.70 ± 0.01*	0.23 ± 0.02$^{\&}$	0.30 ± 0.01*
Brain	SOD (U/mg protein)	10.20 ± 1.20	12.30 ± 1.30*	3.02 ± 1.00$^{\&}$	5.49 ± 0.60*
	CAT (pmol/mg protein)	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01$^{\#}$	0.07 ± 0.01*
	Gpx (U/mg protein)	0.10 ± 0.01	0.15 ± 0.01*	0.07 ± 0.01$^{\&}$	0.13 ± 0.01*

Values represent mean ± SD. Statistical significance respect to the untreated rat is indicated by *$p < 0.001$, **$p < 0.01$. Comparison of SHR vs. WKY rats is indicated by $^{\&}p < 0.001$, $^{\#}p < 0.01$.

as *N*-acetyl-l-cysteine known to have direct antioxidant properties (9). Furthermore, in heart of rat with left ventricular aneurysm, ACE inhibition attenuated oxidative stress and prevented left ventricular remodelling (20).

Lisinopril was proposed as an antioxidant when it was described as an effective inhibitor of copper-induced red cell lipid peroxidation and copper-promoted methemoglobin formation (8).

It is known that Ang II formation decreases by ACE inhibition, whereas levels of other metabolites of the RAS cascade such as Ang-(1–7) and bradykinin increase (29). Since losartan did not modify the parameters of oxidative stress in WKY (22) but lisinopril did, present results provide evidence that kinins and the RAS are involved not only in the hypertensive but also in the normotensive group. In accordance with our results, Cellier *et al*. reported that during ACE inhibition bradykinin inhibits the phosphorylation of ERK1/2 as well as oxidative stress in glomeruli of streptozotocin-diabetic rats (5). These inhibitory actions in the glomeruli are consistent with a renoprotective action of bradykinin and kinin B_2 receptor during diabetes mellitus and support the existence of a role for this autacoid in the beneficial effects of ACE inhibition (5).

Several stimuli are known to activate NAD(P)H oxidases such as cytokines, thrombin, tyrosine kinases, mechanical forces, hyperglycemia, and Ang II via AT_1 receptor signaling. Thus, the inhibition of the AT_1 receptor or the reduced Ang II formation after ACE inhibition will target oxidative stress at its source by preventing the formation of superoxide anion and other ROS that follow NAD(P)H activation (7). Moreover, ACE inhibition enhances NO formation through kinin accumulation and abrogate a number of downstream effects, such as increased cell proliferation, reduced NO bioactivity, lipid peroxidation, resulting from NAD(P)H oxidase activation, and the subsequent ROS formation. NO also inhibits the activity of NAD(P)H oxidase, thereby reducing ROS formation (7) and upregulates vascular extracellular SOD (11). Furthermore, compelling evidence suggests that the kinin B_2 receptor is organ-protective and partakes in the therapeutic effects of ACE inhibition and Ang AT_1 receptor antagonists. Benefits derive primarily from vasodilatory, antihypertensive, antiproliferative, antihypertrophic, antifibrotic, antithrombotic, and antioxidant properties of kinin B_2 receptor activation. Mechanisms include the formation of NO and prostacyclin and the inhibition of NAD(P)H oxidase involving classical and novel signaling pathways (7).

In conclusion, we demonstrated in this work that lisinopril administration diminishes levels of oxidation markers and increases the activity of the antioxidant enzymes not only in the hypertensive SHR group, but also in the normotensive WKY group, thus improving the antioxidant system and protecting the cellular integrity. Therefore, our findings support the hypothesis that ACE inhibition widens the possibilities to lessen ROS-promoted tissue damage, thus suggesting a new therapeutic strategy to attenuate complications associated with oxidative stress. Our data are consistent with the view that a normal regulation of the RAS cascade may be of clinical relevance to treat hypertension and other cardiovascular diseases. Studies in humans testing the hypothesis that lisinopril has antioxidant function *in vivo* are warranted.

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ABBREVIATIONS

ACE, angiotensin-converting enzyme; Ang, angiotensin; CAT, catalase; GSH, reduced glutathione; Gpx, glutathione peroxidase; RAS, renin–angiotensin system; ROS, reactive oxygen species; SHR, spontaneously hypertensive rats; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; WKY, Wistar Kyoto

APPENDIX

Notes

1. Chemicals

NADPH, reduced glutathione (GSH), 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid, glutathione reductase, lisinopril, and *tert*-butyl hydroperoxide were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were of analytical grade.

2. Animals and treatments

Experiments were performed in 12-week-old male SHR and WKY rats (body weight, 372 ± 9 g). The animals were housed in individual cages in a room maintained at 25 ± 2°C on a 12-h light/dark cycle and had free access to standard rat chow and tap water.

One group of SHR (8 animals) and another of WKY (8 animals) were given the ACE inhibitor lisinopril (20 mg/kg/day) in the drinking water during 14 days, (SHR-Lis) and (WKY-Lis), respectively. Other groups of WKY and SHR without treatment were used as controls.

3. Enzyme preparations and assays

Rats were decapitated and kidneys, liver, and brain were excised, washed with an ice-cold saline solution (0.9% NaCl), and homogenized in a Potter–Elvehjem homogenizer. SOD, CAT, and Gpx activities were determined spectrophotometrically in tissue homogenates prepared in a medium containing 140 m*M* KCl and 25 m*M* potassium phosphate buffer (pH 7.4), and centrifuged at 600 *g* for 10 min. The supernatant, a suspension of preserved organelles, was used as homogenate. CAT activity was determined by measuring the decrease in absorbance at 240 nm (6). Gpx activity was assayed by following NADPH oxidation at 340 nm; one unit of the enzyme represents the decrease of 1 mmol of NADPH/min under assay conditions (10). SOD activity was determined by inhibition of adrenochrome formation rate at 480 nm (19). One unit in the SOD assay is defined as the amount of enzymatic protein required to inhibit 50% epinephrine autooxidation.

4. Lipid peroxidation

Lipid peroxidation was determined by measuring the rate of production of TBARS (expressed as malondialdehyde equivalents). One volume of tissue homogenate was mixed with 0.5 volume of trichloroacetic acid (15% wt/vol) and centrifuged at 2,000 *g* for 10 min. Supernatant (1 ml) was mixed with 0.5 ml thiobarbituric acid (0.7% wt/vol) and boiled for 10 min. After cooling, sample absorbance was determined spectrophotometrically at 535 nm. Malondialdehyde concentration was calculated using an value of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$(2).

5. Reduced glutathione

Reduced glutathione (GSH) was determined as nonprotein in rat tissues using the method by Sedlak and Lindsay (26). Briefly, tissues were homogenized in 5.0 ml of cold KCl (1.15%), and proteins were precipitated with trichloroacetic acid. The reaction mixture contained 0.5 ml supernatant, 2.0 ml Tris-EDTA buffer (pH 8.9), and 0.1 ml 6 m*M* DTNB. Absorbance was determined at 412 nm. The results were expressed in μmol GSH mg^{-1} protein.

6. Protein determination

Protein concentration was evaluated by the method of Lowry *et al*. (17) using bovine serum albumin as standard.

7. Statistics

Values in the table and figures are expressed as mean ± S.D. Differences between WKY, SHR, WKY-Lis and SHR-Lis groups were analyzed using two-way ANOVA, considering $p < 0.05$ as statistically significant.

REFERENCES

1. Aizawa T, Ishizaka N, Usui SI, Ohashi N. Ohno M, and Nagai R. Angiotensin II and catecholamines increase plasma levels of 8-epi prostaglandin F_{2a} with different pressor dependencies in rats. *Hypertension* 39: 149–154, 2002.
2. Buege A and Aust SD. Microsomal lipid peroxidation. *Method Enzymol* 52: 302–310, 1978.
3. Busserolles J, Zimowska W, Rock E, Rayssiguier Y, and Mazur A. Rats fed a high sucrose diet have altered heart antioxidant enzyme activity and gene expression. *Life Sci* 71: 1303–1312, 2002.
4. Cavanagh EMV, Inserra F, Ferder L, and Fraga CG. Enalapril and captopril enhance glutathione-dependent antioxidant defenses in mouse tissues. *Am J Physiol Regul Integr Comp Physiol* 278: R572–R577, 2000.
5. Cellier E, Mage M, Duchêne J, Pécher C, Couture R, and Bascands JL. Bradykinin reduces growth factor-induced glomerular ERK1/2 phosphorylation. *Am J Physiol Renal Physiol* 284: F282–F292, 2003.
6. Chance B, Sies H, and Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiol Rev* 59: 527–605, 1979.
7. Couture R and Girolami JP. Putative roles of kinin receptors in the therapeutic effects of angiotensin 1-converting enzyme inhibitors in diabetes mellitus. *Eur J Pharmacol* 500: 467–485, 2004.
8. Fernandes AC, Filipe PM, Freitas JP, and Manso CF. Different effects of thiol and nonthiol ACE inhibitors on copper-induced lipid and protein oxidative modification. *Free Radic Biol Med* 20: 507–514, 1996.
9. Fiordaliso F, Cuccovillo I, Bianchi R, Bai A, Doni M, Salio M, De Angelis N, Ghezzi P, Latini R, and Masson S. Cardiovascular oxidative stress is reduced by an ACE inhibitor in a rat model of streptozotocin-induced diabetes. *Life Sci* 79: 121–129, 2006.
10. Flohé L and Gunzler WA. Assays of glutathione peroxidase. *Method Enzymol* 105: 114–121, 1984.
11. Fukai T, Siegfried MR, Ushio–Fukai M, Cheng Y, Kojda G, and Harrison DG. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise. *J Clin Invest* 105: 1631–1639, 2000.
12. Griendling KK, Minieri CA, Ollerenshaw JD, and Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 74:1141–1148, 1994.
13. Griendling KK and Ushio–Fukai M. Reactive oxygen species as mediators of angiotensin II signaling. *Regul Pept* 91: 21–27, 2000.
14. Lassegue B and Griendling KK. Reactive oxygen species in hypertension. An update. *Am J Hypertens* 17: 852–860, 2004.
15. Lazaro A, Gallego–Delgado J, Justo P, Esteban V, Osende J, Mezzano S, Ortiz A, and Egido J. Long-term blood pressure control prevents oxidative renal injury. *Antioxid Redox Signal* 7: 1285–1293, 2005.
16. Levy B. How to explain the differences between renin angiotensin system modulators. *Am J Hypertens* 18: 134–141, 2005.
17. Lowry HO, Rosebrough NJ, Farr AL, and Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265–275, 1951.
18. Miatello RM, Damiani MT, and Nolly HL. Cardiovascular kinin-generating capability in hypertensive fructose-fed rats. *J Hypertens* 16: 1273–1277, 1998.
19. Misra HP and Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 247: 3170–3175, 1972.
20. Nomoto T, Nishina T, Miwa S, Tsuneyoshi H, Maruyama I, Nishimura K, and Komeda M. Angiotensin-converting enzyme inhibitor helps prevent late remodeling after left ventricular aneurysm repair in rats. *Circulation* 106: 1115–1119, 2002.
21. Polizio AH, Gorzalczany S, Taira C, and Peña C. Aortic coarctation induces oxidative stress in rat tissues. *Life Sci* 79: 596–600, 2006.
22. Polizio AH and Peña C. Effects of angiotensin II type 1 receptor blockade on the oxidative stress in spontaneously hypertensive rat tissues. *Regul Pept* 128: 1–5, 2005.
23. Roberts CK, Vaziri ND, Zhenmin N, and James Bernard R. Correction of long-term diet-induced hypertension and nitrotyrosine accumulation by diet modification. *Atherosclerosis* 163: 321–327, 2002.
24. Rubanyi GM and Vanhoutte PM. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol* 250: H822–827, 1986.
25. Schnackenberg CG, Welch WJ, and Wilcox CS. Normalization of blood pressure and renal vascular resistance in SHR with a membrane-permeable superoxide dismutase mimetic role of nitric oxide. *Hypertension* 32: 59–64, 1998.
26. Sedlak M and Lindsay R. Estimation of total, protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 25: 192–205, 1968.
27. Sindhu RK, Roberts CK, Ehdaie A, Zhan Chang–De, and Vaziri ND. Effects of aortic antioxidant enzymes and NADPH oxidase protein expression. *Life Sci* 76: 945–953, 2005.
28. Vaziri ND, Liang K, and Ding Y. Increased nitric oxide inactivation by reactive oxygen species in lead-induced hypertension. *Kidney Int* 56: 1492–1498, 1999.
29. Yamada K, Iyer SN, Chappell MC, Ganten D, and Ferrario CM. Converting enzyme determines plasma clearance of angiotensin-(1–7). *Hypertension* 32: 496–502, 1998.
30. Zalba G, Beaumont FJ, Gorka SJ, Fortuño A, Fortuño MA, Etayo JC, and Díez J. Vascular NADH/NADPH oxidase is involved in enhanced superoxide production in spontaneously hypertensive rats. *Hypertension* 35: 1055–1061, 2000.

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