Title

Salt-sensitive hypertension in mitochondrial superoxide dismutase deficiency is associated with intra-renal oxidative stress and inflammation

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ORIGINAL ARTICLE

Salt-sensitive hypertension in mitochondrial superoxide dismutase deficiency is associated with intra-renal oxidative stress and inflammation

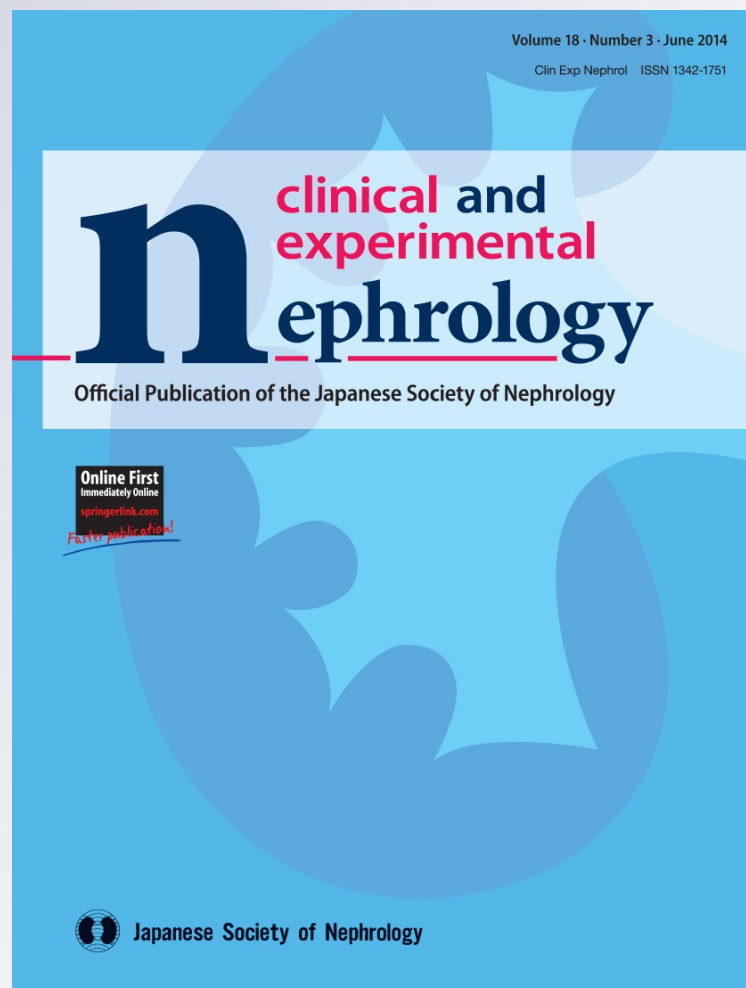**Kyubok Jin · Nosratola D. Vaziri**

Abstract

Background Renal interstitial inflammation and oxidative stress are invariably present and play a key role in the pathogenesis of hypertension in experimental animals. Mitochondria are the major source of reactive oxygen species (ROS). ROS generated in the mitochondria are normally contained by the mitochondrial antioxidant system including manganese superoxide dismutase (MnSOD). We have previously shown that a high salt diet causes hypertension in MnSOD-deficient ($MnSOD^{+/-}$) mice but not in wild-type mice. The present study was undertaken to determine the effect of a high salt diet on oxidative and inflammatory pathways in the kidneys of $MnSOD^{+/-}$ mice compared to the wild-type mice.

Methods Wild-type ($MnSOD^{+/+}$) and $MnSOD^{+/-}$ mice were randomized to receive a regular or a high salt diet for 4 months. Tail arterial pressure was measured and timed urine collection was obtained. The animals were then euthanized and the kidneys were harvested and processed for histological examination and Western blot analyses.

Results In confirmation of our earlier study, a high salt diet resulted in a significant rise in arterial pressure and urinary albumin excretion in $MnSOD^{+/-}$ mice. This was accompanied by upregulation of NAD(P)H oxidase subunits, activation of nuclear factor kappa B, and elevation of PAI-1, iNOS, oxidized LDL receptor, and CD36 in the kidneys of the $MnSOD^{+/-}$ mice fed the high salt diet. In contrast, consumption of a high salt diet did not significantly alter blood pressure, urine protein excretion, or the measured oxidative and inflammatory mediators in the wild-type mice.

Conclusion Salt-induced hypertension in $MnSOD^{+/-}$ mice is associated with activation of intra-renal inflammatory and ROS generating pathways.

Keywords Mitochondria · Oxidative stress · Inflammation · Superoxide dismutase

Introduction

Mitochondria are the major source of cellular superoxide production in aerobic organisms [1]. Superoxide produced by mitochondria is converted by mitochondrial superoxide dismutase (MnSOD) to hydrogen peroxide which is, in turn, converted to water by catalase and glutathione peroxidase. MnSOD deficiency leads to elevation of uncontained superoxide which can cause oxidative stress, inflammation, and tissue injury. In fact, homozygous MnSOD-deficient ($MnSOD^{-/-}$) mice die of dilated cardiomyopathy, fatty liver, and numerous other complications within days after birth. However, heterozygous mice with partial MnSOD deficiency ($MnSOD^{+/-}$) survive but show evidence of superoxide-induced mitochondrial damage and senescence [2]. Earlier studies have shown that a high salt diet causes hypertension in MnSOD-deficient ($MnSOD^{+/-}$) mice but not in wild-type mice [3]. Oxidative stress and renal interstitial inflammation are invariably present and play a major role in elevation of arterial pressure in animal models of genetic and acquired hypertension. Oxidative stress in the kidney and vascular tissues can raise arterial pressure by several mechanisms including

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superoxide-mediated inactivation and impaired production of nitric oxide [4] and accumulation of pro-inflammatory, vasoconstrictive, and anti-natriuretic isoprostanes, among others [5]. In addition by activating the redox-sensitive nuclear factor kappa B (NFκB), oxidative stress promotes interstitial inflammation and ectopic generation of intra-renal angiotensin II (AngII) by infiltrating macrophages as shown in animal models of hypertension [6–8]. Activation of angiotensin receptor type 1 (AT1), in turn, amplifies superoxide production and oxidative stress by activating NAD(P)H oxidase. Together these events work in harmony to promote hypertension.

Renal tubular epithelial cells are rich in mitochondria which are essential for generation of adenosine triphosphate (ATP) to accommodate reabsorption of filtered sodium (Na) by the Na/K ATPase pump. Since generation of ATP in the mitochondria is coupled with production of superoxide, deficiency of MnSOD which catalyzes conversion of this highly reactive free radical to hydrogen peroxide can render the kidney susceptible to oxidative stress. In this context increased Na load and the associated increase in energy requirement for tubular Na transport can lead to intra-renal oxidative stress and inflammation in MnSOD-deficient animals. In an earlier study we found that a high salt diet causes hypertension in MnSOD-deficient ($MnSOD^{+/-}$) mice but not in wild-type mice. The present study was undertaken to determine the effect of a high salt diet on intra-renal oxidative and inflammatory pathways in the $MnSOD^{+/-}$ mice compared to wild-type mice.

Methods

Study groups

MnSOD-deficient ($CD1/MnSOD^{+/-}$) mice were used in the study. Age-matched wild-type CD1 ($MnSOD^{+/+}$) mice served as controls. The animals were fed regular rodent chow (Purina Mills, ProLab® RMH 2500, LabDiet, Brentwood, MO, USA) ad libitum. The animals were housed in a climate-controlled, pathogen-free environment with a 12:12 h light–dark cycle. The animals used in this study were derived from a colony developed and maintained by Dr. Wallace's laboratory in the Department of Biological Chemistry, University of California, Irvine, CA, USA [2, 9]. The study protocol was approved by the Animal Care and Use Committee of the University of California.

The MnSOD-deficient and wild-type mice were randomized into high-salt and regular-salt groups. The animals assigned to the high-salt group were fed a diet containing 4 % sodium chloride (Harlan Teklad,

TD.92034 laboratory diet) for 16 week. The animals assigned to the regular salt group received a regular diet containing 0.4 % sodium chloride. Each subgroup contained six to seven animals.

Blood pressure was determined by tail cuff plethysmography (CODA2, Kent Scientific Corporation, Torrington, CT, USA) at 0, 4, 8, 12 and 16 weeks. Conscious mice were placed in a restrainer on a warming pad and allowed to rest inside the cage for 15 min before the blood pressure measurements. The mouse tail was placed inside a tail cuff, and the cuff was inflated and released several times to allow the animal to be conditioned to the procedure. On each occasion tail blood pressure was measured 4 times and the average of the values obtained was used. At the end of the 16-week observation period the rats were placed in metabolic cages for a 24-h urine collection. The animals were then anesthetized (sodium pentobarbital, 50 mg/kg i.p.) and euthanized by exsanguination using cardiac puncture. The kidneys, heart, and blood were removed and kidneys and heart weighed. A piece of the kidney was separated and fixed in 10 % formalin for histological examination. The remaining tissue was cleaned with phosphate-buffered saline, snap-frozen in liquid nitrogen, and stored at -70 °C until processing.

Biochemical determinations

The urine albumin concentration was measured using a kit purchased from Chondrex Inc. (Redmond, WA, USA). Plasma urea and albumin and urine and plasma creatinine concentrations were measured using kits purchased from Bioassay Systems (Hayward, CA, USA). Creatinine clearance was calculated using standard equation.

The concentration of nitric oxide (NO) metabolites, nitrate plus nitrite (NOx), in the urine samples were determined by the Sievers Instruments model 270B nitric oxide analyzer (NOA; Sievers Instruments, Boulder, CO, USA) as described in our earlier studies [10]. Urine 8-isoprostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) was measured using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA).

Western blot analyses

Target proteins in the cytoplasmic fractions of the kidney cortex tissue were measured by Western blot analysis as previously described [11, 12] using the following antibodies. The antibodies against $gp91^{phox}$, $p47^{phox}$, $p67^{phox}$, Rac1, iNOS and PAI-1 were purchased from BD Bioscience (San Jose, CA, USA). The antibodies against phospho-IκB (Cell Signaling Technology, Denver, CO, USA) and LOX-1 (Abcam Inc., Cambridge, MA, USA) were purchased from the cited sources. β-actin (Sigma, St.

Louis, MO, USA) was used for the measurement of the housekeeping protein for the cytosolic target proteins.

Briefly, aliquots containing 50 μg of protein were fractionated on 8 and 4–20 % Tris–glycine gel (Novex Inc., San Diego, CA, USA) at 120 V for 2 h, and transferred to Hybond-ECL membrane (Amersham Life Science Inc., Arlington Heights, IL, USA). The membrane was incubated for 1 h in blocking buffer (1× TBS, 0.05 % Tween-20 and 5 % nonfat milk) and then overnight in the same buffer containing the appropriate antibodies. The membrane was washed three times for 5 min in 1× TBS and 0.05 % Tween-20 prior to a 2-h incubation in a buffer (1× TBS, 0.05 % Tween-20 and 3 % nonfat milk) containing horseradish peroxidase-linked anti-rabbit IgG and anti-mouse IgG (Amersham Life Science Inc.) at a 1:1,000 dilution. The membrane was washed four times and developed by autoluminography using the ECL chemiluminescent agents (Amersham Life Science Inc.).

Histological examination

Light microscopy studies were performed in formalin-fixed renal sections stained with periodic acid-Schiff and hematoxylin-eosin stains as described in previous investigations [13, 14]. Following sacrifice, a piece of the kidney was promptly removed and immersed in 4 % paraformaldehyde/phosphate-buffered saline at 4 °C overnight. Thereafter, the tissue was cryoprotected in 30 % sucrose at 4 °C and frozen in liquid nitrogen. The frozen sections were then cut using a Leica CM 1900 UV (Leica, Germany) at 10 μm. Each section was air dried for 1 h and fixed in 10 % formalin for 10 min. An Olympus BX51 System Microscope and DP70 microscope digital camera with Sigma Pro (Leesburgh, VA, USA) software were used for the computer-assisted image analysis.

Statistical analysis

ANOVA and post hoc Tukey tests (SPSS 14.0, Chicago, IL, USA) were used for statistical evaluation of the data, which are presented as the mean ± standard deviation. P values ≤0.05 were considered significant.

Results

General data

Data are summarized in Table 1 and Fig. 1. No significant difference was found in body weight or serum concentrations of urea or albumin among the study groups. As expected, urine volume significantly increased with the high salt diet in both MnSOD-deficient and wild-type mice but more so in the MnSOD-deficient group. Consumption of the high salt diet did not significantly change the weights of either heart (wild-type = 0.28 ± 0.02 g; MnSOD-deficient = 0.23 ± 0.01 g) or the kidneys (wild-type = 0.85 ± 0.04 g; MnSOD-deficient = 0.74 ± 0.05 g) in the study animals.

Urinary excretion of isoprostane was modestly elevated in the MnSOD-deficient mice consuming the regular diet compared with their wild-type counterparts. Consumption of the high salt diet resulted in a marked increase in urinary isoprostane excretion in both groups but more so in the MnSOD-deficient than the wild-type mice. Urinary excretion of NO metabolites (NOx) in the MnSOD-deficient mice consuming regular diet was significantly less than that found in their wild-type counterparts. Consumption of the high salt diet resulted in a marked reduction in urinary NOx in the wild-type mice but did not significantly alter urinary NOx in the MnSOD-deficient mice.

Table 1 General data in wild-type mice and MnSOD-deficient mice fed a regular salt or high salt diet

	Wild-type		MnSOD(+/−)	
	Regular diet (N = 6)	High salt diet (N = 7)	Regular diet (N = 6)	High salt diet (N = 7)
Body weight (g)	50.57 ± 3.50	56.36 ± 3.04	51.82 ± 3.44	54.87 ± 3.13
Plasma urea (mg/dL)	25.77 ± 3.24	25.26 ± 1.55	27.02 ± 3.27	24.63 ± 2.34
Plasma creatinine (mg/dL)	0.15 ± 0.03	0.18 ± 0.05	0.39 ± 0.05	0.27 ± 0.10
Plasma albumin (g/dL)	2.11 ± 0.07	1.99 ± 0.09	1.98 ± 0.14	2.13 ± 0.16
Urine volume (mL/day/100 g)	2.10 ± 0.57	4.80 ± 1.02[c]	2.79 ± 0.34	6.02 ± 0.73[b]
Urinary excretion of sodium (mmol/day/100 g)	0.42 ± 0.11	4.70 ± 0.37[c]	0.71 ± 0.06	9.30 ± 1.27[a,b]
Urinary excretion of albumin (mg/day)	1.84 ± 0.13	5.65 ± 2.92	2.46 ± 0.35	8.67 ± 2.35[a,b]

Values are presented as the mean ± SD

[a] $P < 0.05$ wild-type/high salt vs MnSOD^{+/−}/high salt group

[b] $P < 0.05$ MnSOD^{+/−}/regular salt vs MnSOD^{+/−}/high salt group

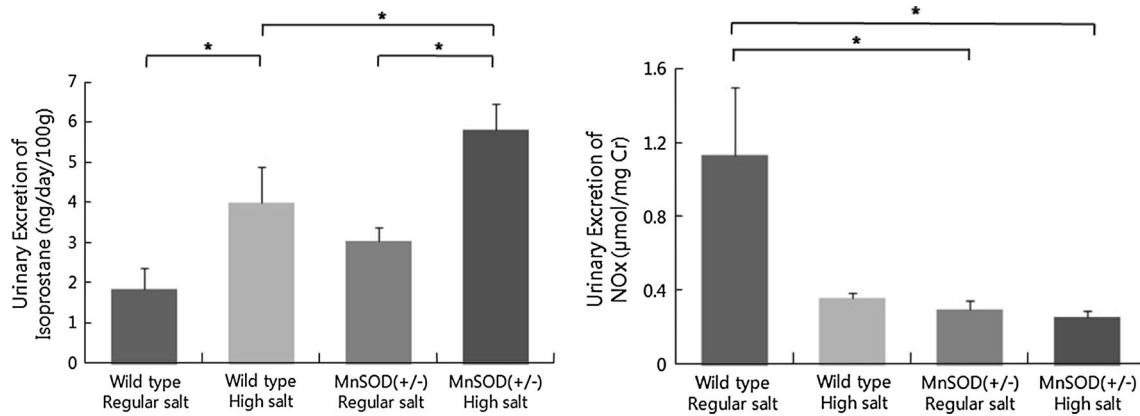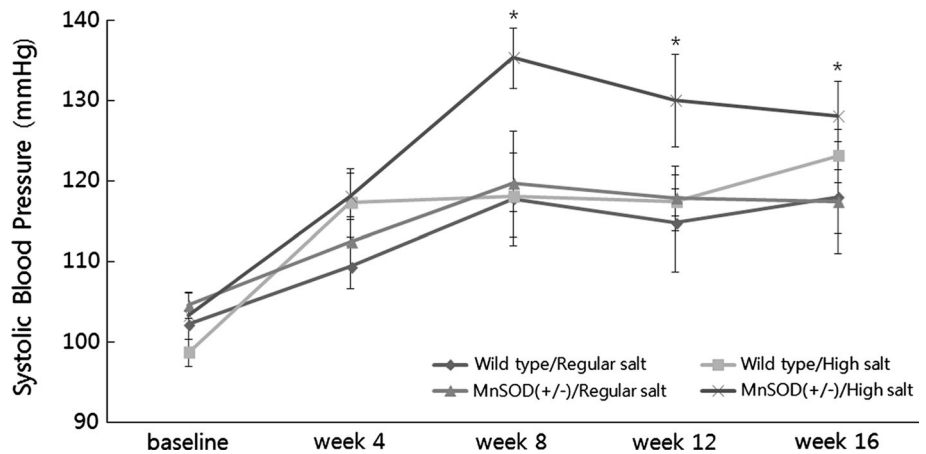[c] $P < 0.05$ wild-type/regular salt vs wild-type/high salt group

Fig. 1 Urinary excretion of F_2-isoprostane and NOx in wild-type and MnSOD$^{+/-}$ mice with a regular or high salt intake

Fig. 2 Changes in systolic blood pressure in wild-type and MnSOD$^{+/-}$ mice during the administration of a regular salt or high salt diet

Blood pressure

The data are shown in Fig. 2. The baseline blood pressure in animals consuming a regular diet was similar among the MnSOD-deficient and wild-type animals. Consumption of the high salt diet resulted in a significant rise in the systolic arterial pressure in the MnSOD-deficient mice. In contrast, the high salt diet did not significantly alter systolic blood pressure in the wild-type mice. This observation points to the presence of salt sensitivity in the MnSOD-deficient mice.

NAD(P)H oxidase data

Data are illustrated in Fig. 3. No significant difference was found in the renal tissue abundance of gp91^{phox}, p47^{phox}, p67^{phox} and Rac1 subunits of NAD(P)H oxidase between the MnSOD-deficient and wild-type mice consuming the regular diet. Consumption of the high salt diet resulted in a significantly increase in gp91^{phox}, p47^{phox}, p67^{phox} and Rac1 abundance in the renal tissue of the MnSOD-deficient mice but not the wild-type mice. As a major source of non-mitochondrial production of superoxide, upregulation of

NAD(P)H oxidase points to its contribution to the salt-induced oxidative stress in this model.

NFkB, PAI-1, iNOS, LOX-1 and histological data

Data are shown in Figs. 4 and 5. No significant difference was found in the renal tissue abundance of pIκB, PAI-1, iNOS or LOX-1 abundance between the MnSOD-deficient and wild-type mice consuming the regular diet. Consumption of the high salt diet resulted in a significant increase in pIκB, PAI-1, iNOS and LOX-1 abundance in the renal tissue of the MnSOD-deficient mice but not the wild-type mice. This was associated with renal interstitial accumulation of lymphocytes and macrophages (Fig. 5) as shown in our earlier study [3] pointing to the susceptibility of the MnSOD-deficient mice to high salt diet-induced renal inflammation.

Discussion

In confirmation of our earlier study [3], consumption of the high salt diet resulted in a significant increase in arterial
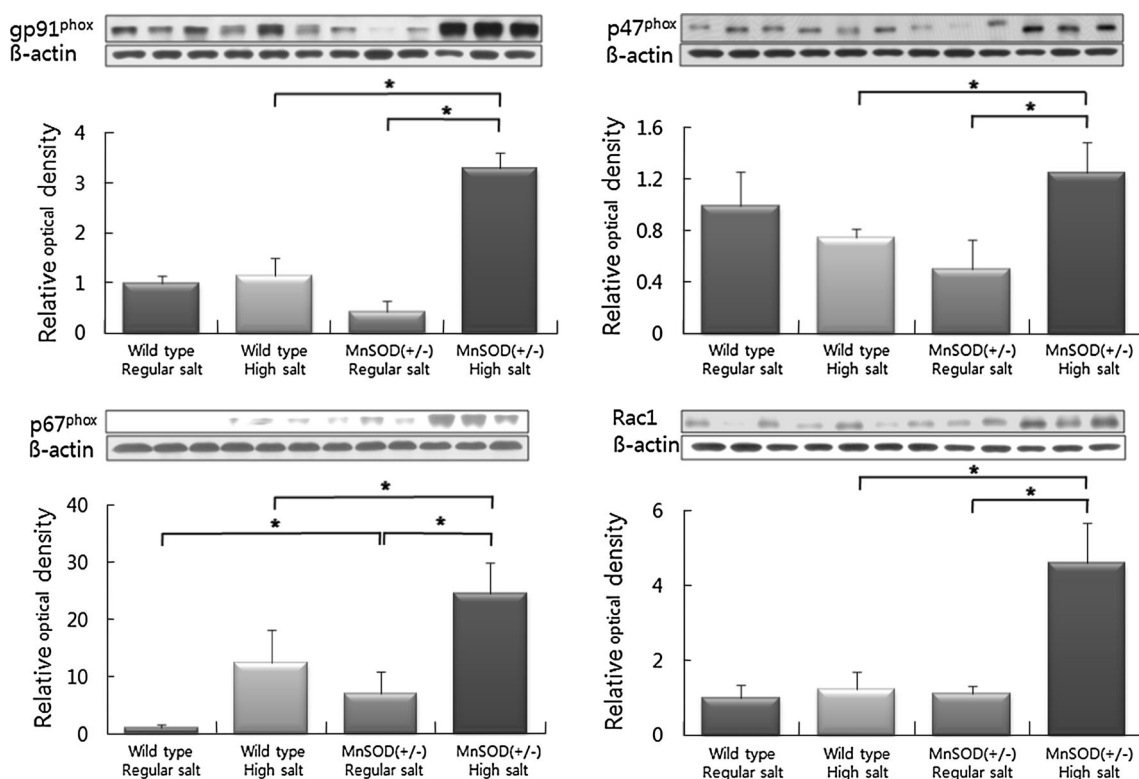
Fig. 3 Representative Western blot and group data depicting gp91^{phox}, p47^{phox}, p67^{phox}, Rac1, and ß-actin abundance in the kidney cortex of wild-type and MnSOD$^{+/-}$ mice fed a regular or high salt diet for 16 weeks

pressure in the MnSOD-deficient mice but not in the wild-type mice. This phenomenon illustrates the association of MnSOD deficiency with salt-sensitivity. The rise in arterial blood pressure in response to increased dietary salt intake in the MnSOD-deficient mice was accompanied by upregulation of NAD(P)H oxidase which is a major source of non-mitochondrial superoxide production in the renal and cardiovascular tissues. Upregulation of NAD(P)H oxidase in the kidney and vascular tissues is a common feature of nearly all models of acquired and hereditary hypertension [15–18]. Therefore, finding elevated renal tissue NAD(P)H oxidase in this model is consistent with that observed in other models of hypertension. Upregulation of NAD(P)H oxidase in the kidney tissues of the MnSOD-deficient mice consuming the high salt diet was associated with marked elevation of urinary isoprostane which is a biomarker of oxidative stress and an anti-natriuretic, vasoconstrictive and proinflammatory agent.

Urinary excretion of NO metabolites (NOx) in MnSOD-deficient mice consuming regular diet was significantly lower than their wild-type counterparts and remained low on the high salt diet. These findings point to reduced NO production in the MnSOD-deficient mice. Oxidative stress is known to reduce NO production by uncoupling nitric oxide synthase (NOS), depleting NOS co-factor (tetrahydrobiopterin), and inhibiting dimethylarginine

dimethylaminohydrolase, the enzyme that degrades asymmetrical dimethylarginine, the endogenous competitive inhibitors of NOS. Together these events promote hypertension and tissue injury by reducing eNOS-derived NO production and transforming eNOS from an NO-producing enzyme to a superoxide-producing enzyme thereby amplifying oxidative stress and inflammation [19–21]. Thus the reduction in NO production in MnSOD-deficient mice must be, in part, due to the ambient oxidative stress in these animals.

Consumption of the high salt diet in the wild-type mice resulted in a significant reduction of urinary NOx level denoting the reduction in NO production. Regulation of blood pressure is largely dependent on the interplay of factors influencing the intravascular volume/cardiac output and the balance between the vasoconstrictive and vasodilatory factors. Under normal conditions a high salt diet suppresses the renin−angiotensin system. This helps to reduce vascular resistance by lowering AngII production and mitigate volume expansion by reducing aldosterone level. In addition, suppression of the angiotensin system reduces AngII-mediated superoxide production in the kidney and vascular tissues and thereby enhances NO bioavailability. These events help to reduce the need for NO since there are less vasoconstrictive and sodium retaining forces for NO to counteract. Thus, the reduction in NOx in
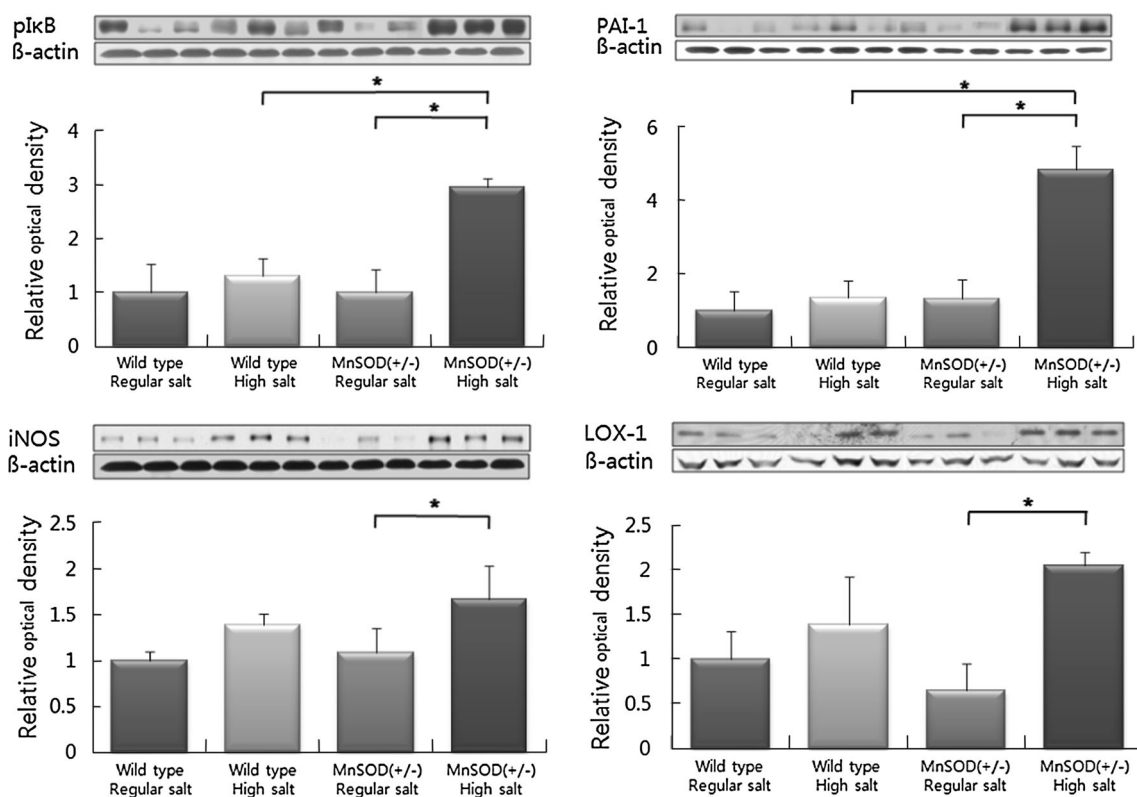
Fig. 4 Representative Western blot and group data depicting pIκB, PAI-1, iNOS, LOX-1, and ß-actin abundance in the kidney cortex of wild-type and MnSOD$^{+/-}$ mice fed a regular or high salt diet for 16 weeks
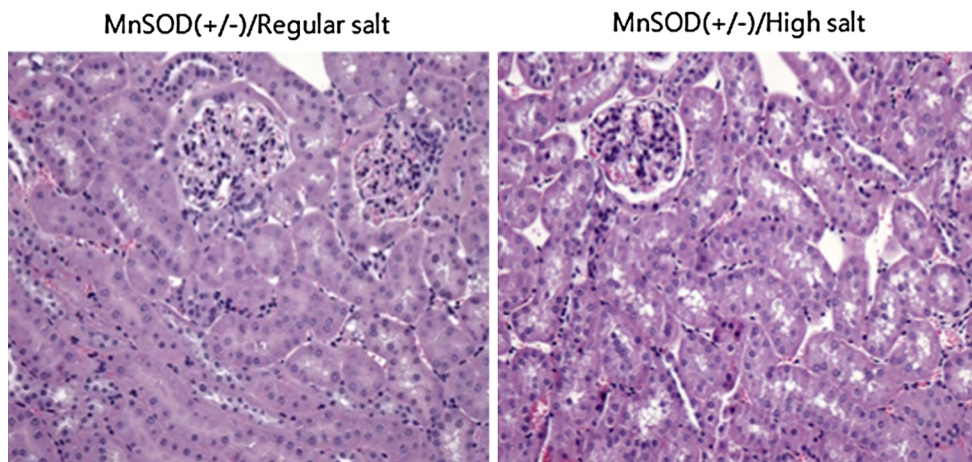

Fig. 5 Renal histology in MnSOD$^{+/-}$ mice fed a regular salt diet and MnSOD$^{+/-}$ mice fed a high salt diet (×200, hematoxylin-eosin staining)

response to the high salt diet in the wild-type group represents an adaptive response.

Urinary NOx excretion in the MnSOD-deficient mice consuming a regular diet was significantly lower than their wild-type counterparts and did not change with consumption of a high salt diet. It should be noted that consumption of a high salt diet resulted in a significant upregulation of iNOS in the kidneys of the MnSOD-deficient mice, reflecting activation of inflammatory pathway. The lack of change in NOx level despite upregulation of iNOS signifies a significant decline in endothelial NOS (eNOS)-derived NO in these animals. This was compounded by avid inactivation of NO by mitochondria- and NAD(P)H oxidase-derived superoxide which leads to further decline in NO bioavailability in the MnSOD-deficient mice consuming the high salt diet.

Under physiological conditions the rise in blood pressure increases NO production via shear stress-induced

activation of eNOS [22]. Thus, the failure of MnSOD-deficient mice consuming a high salt diet to raise NO production despite a significant rise in blood pressure represents a maladaptive response.

Oxidative stress in the kidney and vascular tissue causes hypertension [23] and hypertension promotes oxidative stress [24–26], denoting the participation of oxidative stress and hypertension in a vicious circle. Since kidney tissues in the study animals were obtained when hypertension was well established in the MnSOD-deficient mice consuming the high salt diet, it is not possible to determine whether hypertension preceded or followed the onset of oxidative stress in this model.

Renal interstitial inflammation is a common feature of hypertensive disorders and plays a major part in the pathogenesis of hypertension [6, 27–30]. In fact, as shown in our original study, development of hypertension in MnSOD-deficient mice fed a high salt diet was associated with significant renal interstitial infiltration of T lymphocytes and macrophages [3]. Hypertension in the MnSOD-deficient mice consuming a high salt diet was associated with a marked increase in phosphorylated IκB. This phenomenon points to activation of NFκB which is the master regulator of genes encoding inflammatory mediators. As a redox sensitive transcription factor, the observed activation NFκB must be in part mediated by increased release of reactive oxygen species (ROS) from MnSOD-deficient mitochondria and increased production of superoxide due to upregulation of NAD(P)H oxidase. The observed activation of NFκB in the renal tissue of MnSOD-deficient mice with salt-induced hypertension was associated with interstitial accumulation of inflammatory cells in the kidney confirming our earlier studies [3]. Activation of the oxidative and inflammatory pathways in the renal tissues of the study animals was accompanied by upregulation of iNOS, PAI-1, and LOX-1 which participate in the development of chronic kidney disease by promoting fibrosis, protein nitrosylation and foam cell formation.

In an elegant study, Dikalova et al. [31] found a marked reduction of arterial pressure with administration of the mitochondria-targeted SOD-mimetic agent, mitoTEMPO, in mice with AngII-induced hypertension as well as mice with DOCA/salt-induced hypertension. In vitro studies revealed significant increase in mitochondrial and cellular superoxide production and reduced NO production in AngII-treated endothelial cells. AngII-induced increase in superoxide production and reduced NO production were reversed by pre-treatment of cells with mitoTEMPO. The effects of mitoTEMPO were simulated by over-expression of the mitochondrial MnSOD (but not with the administration of non-targeted TEMPOL). In contrast MnSOD depletion with small interfering RNA increased both basal and AngII-stimulated superoxide production. The findings

of the present study in MnSOD-deficient mice are consistent with those of Dikalova et al. and illustrate the role of mitochondrial SOD deficiency/dysfunction and increased mitochondrial superoxide production in the pathogenesis of hypertension.

In conclusion, hypertension and renal interstitial inflammation in MnSOD-deficient mice consuming a high salt diet is associated with the upregulation of superoxide-generating enzyme, NAD(P)H oxidase, activation of NFκB and interstitial inflammation.

References

1. Esposito LA, Melov S, Panov A, et al. Mitochondrial disease in mouse results in increased oxidative stress. Proc Natl Acad Sci USA. 1999;96(9):4820–5.
2. Kokoszka JE, Coskun P, Esposito LA, et al. Increased mitochondrial oxidative stress in the Sod2 (+/−) mouse results in the age-related decline of mitochondrial function culminating in increased apoptosis. Proc Natl Acad Sci USA. 2001;98(5):2278–83.
3. Rodriguez-Iturbe B, Sepassi L, Quiroz Y, et al. Association of mitochondrial SOD deficiency with salt-sensitive hypertension and accelerated renal senescence. J Appl Physiol. 2007;102(1):255–60.
4. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res. 2000;87(10):840–4.
5. Romero JC, Reckelhoff JF. State-of-the-Art lecture. Role of angiotensin and oxidative stress in essential hypertension. Hypertension. 1999;34(4):943–9.
6. Franco M, Martínez F, Quiroz Y, et al. Renal angiotensin II concentration and interstitial infiltration of immune cells are correlated with blood pressure levels in salt-sensitive hypertension. Am J Physiol Regul Integr Comp Physiol. 2007;293(1):R251–6.
7. Franco M, Sanchez-Lozada LG, Bautista R, et al. Pathophysiology of salt-sensitive hypertension: a new scope of an old problem. Blood Purif. 2008;26(1):45–8.
8. Rodriguez-Iturbe B, Johnson RJ. The role of renal microvascular disease and interstitial inflammation in salt-sensitive hypertension. Hypertens Res. 2010;33(10):975–80.
9. Melov S, Coskun P, Patel M, et al. Mitochondrial disease in superoxide dismutase 2 mutant mice. Proc Natl Acad Sci USA. 1999;96(3):846–51.
10. Vaziri ND, Ni Z, Wang XQ, et al. Downregulation of nitric oxide synthase in chronic renal insufficiency: role of excess PTH. Am J Physiol. 1998;274(4):F642–9.
11. Kim HJ, Vaziri ND. Sterol regulatory element-binding proteins, liver X receptor, ABCA1 transporter, CD36, scavenger receptors A1 and B1 in nephrotic kidney. Am J Nephrol. 2009;29(6):607–14.
12. Kim HJ, Moradi H, Yuan J, et al. Renal mass reduction results in accumulation of lipids and dysregulation of lipid regulatory proteins in the remnant kidney. Am J Physiol Ren Physiol. 2009;296(6):F1297–306.
13. Alvarez V, Quiroz Y, Nava M, et al. Overload proteinuria is followed by salt-sensitive hypertension caused by renal infiltration of immune cells. Am J Physiol Ren Physiol. 2002;283(5):F1132–41.
14. Quiroz Y, Ferrebuz A, Romero F, et al. Melatonin ameliorates oxidative stress, inflammation, proteinuria, and progression of

renal damage in rats with renal mass reduction. Am J Physiol Ren Physiol. 2008;294(2):F336–44.

15. Montezano AC, Touyz RM. Oxidative stress, Noxs, and hypertension: experimental evidence and clinical controversies. Ann Med. 2012;44:S2–16.

16. Luo Z, Teerlink T, Griendling K, et al. Angiotensin II and NADPH oxidase increase ADMA in vascular smooth muscle cells. Hypertension. 2010;56(3):498–504.

17. Nishi EE, Oliveira-Sales EB, Bergamaschi CT, et al. Chronic antioxidant treatment improves arterial renovascular hypertension and oxidative stress markers in the kidney in Wistar rats. Am J Hypertens. 2010;23(5):473–80.

18. Tian N, Moore RS, Phillips WE, et al. NADPH oxidase contributes to renal damage and dysfunction in Dahl salt-sensitive hypertension. Am J Physiol Regul Integr Comp Physiol. 2008;295(6):R1858–65.

19. Vaziri ND, Rodríguez-Iturbe B. Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension. Nat Clin Pract Nephrol. 2006;2(10):582–93.

20. Förstermann U, Münzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation. 2006;113(13):1708–14.

21. Sydow K, Münzel T. ADMA and oxidative stress. Atheroscler Suppl. 2003;4(4):41–51.

22. Ungvari Z, Sun D, Huang A, et al. Role of endothelial [Ca2+]i in activation of eNOS in pressurized arterioles by agonists and wall shear stress. Am J Physiol Heart Circ Physiol. 2001;281(2):H606–12.

23. Vaziri ND, Ni Z. Expression of NOX-I, gp91phox, p47phox and P67phox in the aorta segments above and below coarctation. Biochim Biophys Acta. 2005;1723(1–3):321–7.

24. Sindhu RK, Roberts CK, Ehdaie A, et al. Effects of aortic coarctation on aortic antioxidant enzymes and NADPH oxidase protein expression. Life Sci. 2005;76(8):945–53.

25. Barton CH, Ni Z, Vaziri ND. Enhanced nitric oxide inactivation in aortic coarctation-induced hypertension. Kidney Int. 2001;60(3):1083–7.

26. Vaziri ND, Wang XQ, Oveisi F, et al. Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. Hypertension. 2000;36(1):142–6.

27. Rodríguez-Iturbe B, Franco M, Tapia E, et al. Renal inflammation, autoimmunity and salt-sensitive hypertension. Clin Exp Pharmacol Physiol. 2012;39:96–103.

28. Guzik TJ, Hoch NE, Brown KA, et al. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. J Exp Med. 2007;204:2449–60.

29. Rodríguez-Iturbe B, Quiroz Y, Nava M, et al. Reduction of renal immune cell infiltration results in blood pressure control in genetically hypertensive rats. Am J Physiol Renal Physiol. 2002;282:F191–201.

30. Harrison DG, Guzik TJ, Lob HE, et al. Inflammation, immunity, and hypertension. Hypertension. 2011;57:132–40.

31. Dikalova AE, Bikineyeva AT, Budzyn K, et al. Therapeutic targeting of mitochondrial superoxide in hypertension. Circ Res. 2010;107(1):106–16.