Association of mitochondrial SOD deficiency with salt-sensitive hypertension and accelerated renal senescence

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Rodriguez-Iturbe B, Sepassi L, Quiroz Y, Ni Z, Vaziri ND. Association of mitochondrial SOD deficiency with salt-sensitive hypertension and accelerated renal senescence. J Appl Physiol 102: 255–260, 2007. First published October 5, 2006; doi:10.1152/japplphysiol.00513.2006.—Mitochondria are the major source of superoxide (O2•−) in the aerobic organisms. O2•− produced by the mitochondria is converted to hydrogen peroxide by mitochondrial superoxide dismutase (SOD2). Mice with complete SOD2 deficiency (SOD2−/−) exhibit dilated cardiomyopathy and fatty liver leading to neonatal mortality, whereas mice with partial SOD2 deficiency (SOD2+/−) show evidence of O2•−-induced mitochondrial damage resembling cell senescence. Since earlier studies have provided compelling evidence for the role of oxidative stress and tubulointerstitial inflammation in the pathogenesis of hypertension, we tested the hypothesis that partial SOD2 deficiency may result in hypertension. Wild-type (SOD2+/+) and partial SOD2-deficient (SOD2+/−) mice had similar blood pressures at 6–7 mo of age, but at 2 yr SOD2−/− mice had higher blood pressure. Oxidative stress, renal interstitial T-cell and macrophage infiltration, tubular damage, and glomerular sclerosis were all significantly increased in 2-yr-old SOD2+/− mice. High-salt diet induced hypertension in 6-mo-old SOD2-deficient mice but not in wild-type mice. In conclusion, partial SOD2 deficiency results in oxidative stress and renal interstitial inflammation, changes compatible with accelerated renal senescence and salt-sensitive hypertension. These findings are consistent with the pattern described in numerous other models of salt-sensitive hypertension and resemble that commonly seen in elderly humans.

O2•− is converted to hydrogen peroxide by a family of enzymes known as superoxide dismutase (SOD). SOD is present as three different isoforms: CuZnSOD (SOD1), which is predominantly located in the cytoplasm; MnSOD isoform (SOD2), which is located in the mitochondria; and the extra-cellular SOD (SOD3 or EC-SOD), which is bound to heparan sulfate proteoglycans in the extracellular space. Previous studies have demonstrated that nonselective pharmacological inhibition of SOD worsens (14), whereas administration of SOD-mimetic agents ameliorate (4) oxidative stress and hypertension in spontaneously hypertensive rats. Studies of the SOD1-knockout mice (CuZnSOD−/−) consuming regular diet showed impaired arterial vasorelaxation but no hypertension at young age (6). Similarly, young SOD3-knockout mice consuming regular diet were found to have normal baseline blood pressure but an exaggerated hypertensive response to angiotensin II infusion and surgically induced unilateral renal artery stenosis (9).

To our knowledge, the role of mitochondrial SOD (SOD2) in the regulation of arterial blood pressure has not been evaluated. This is, in part, due to the fact that, unlike cytoplasmic or extracellular SOD deficiencies, which have no effect on animal viability, complete SOD2 deficiency in mice results in dilated cardiomyopathy, fatty liver, and numerous other abnormalities culminating in neonatal lethality (12). Studies of the heterozygote SOD2m1Cje mice (SOD2+/−) have shown evidence of O2•−-induced mitochondrial damage resembling cellular senescence (9). Relevant to the present work is the fact that the aging process is associated with a rise in average blood pressure and commonly leads to development of salt-sensitive hypertension in humans (30).

Several studies from our laboratories (1, 17, 19, 20, 22, 23, 25) demonstrated the role of oxidative stress and renal interstitial inflammation in the pathogenesis and maintenance of salt-sensitive hypertension. The present study was designed to test the hypothesis that partial SOD2 deficiency may lead to renal interstitial inflammation and salt-sensitive hypertension.

METHODS

Animals. Six-month-old and 2-yr-old SOD2-deficient (CD1/SOD2+/−) mice were used in the study. Age-matched CD (SOD2+/+) mice served as controls. The animals were fed regular rodent chow (Purina Mills, ProLab RMH 2500 laboratory diet) ad libitum. The animals were housed in a climate-controlled, pathogen-free environment with 12:12-h light-dark cycles. The animals used in the study were derived from a colony developed and maintained by Dr. Wallace’s laboratory, Biological Chemistry, University of California, Irvine, CA (9, 12). The study protocol was approved by the Animal Care and Use Committee of the University of California, Irvine.

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The 6-mo-old animals were randomized into high-salt and normal-salt groups. The animals assigned to the high-salt group were fed a diet containing 2% sodium chloride for 4 wk. The animals randomized to the normal salt group received regular diet. Arterial blood pressure was measured by tail plethysmography (Harvard Apparatus Holliston) using a mouse tail cuff as described previously (18). Six to eight animals were used in each subgroup. At the conclusion of the observation period, under general anesthesia (Nembutal 50 mg/kg ip), the animals were euthanized by exsanguination via cardiac puncture. Kidneys were harvested and fixed in 10% formalin and processed for histological and immuno-histological evaluations.

**Plasma malondialdehyde assay.** Plasma malondialdehyde assay was measured by HPLC as described in our laboratory’s earlier studies (29).

Plasma urea concentration was measured with QuantiChrom Urea assay kit DIUR 500 (BioAssay Systems, Hayward, CA).

**Histology and immunohistology.** The left kidney was used for histological studies. Kidney tissues were fixed in 10% buffered formalin, embedded in paraffin, and cut in 3- to 4-μm sections that were stained with periodic acid-Schiff and hematoxylin and eosin for light microscopy studies. Glomerular and tubulointerstitial damage were evaluated as described in previous communications (1, 17, 21). Briefly, glomeruli were graded from 0 to +4: grade 0 = normal, grade 1 = <25%, grade 2 = 25–50%, grade 3 = 50–75%, and grade 4 = sclerosis occupying >75% of the glomerular tuft. The glomerulosclerosis score was obtained as follows: \( [(1 \times n \text{ glomeruli with} +1) + (2 \times n \text{ glomeruli with} +2) + (3 \times n \text{ glomeruli with} +3) + (4 \times n \text{ glomeruli with} +4)] \times 100/\text{total number of glomeruli} \). All glomeruli suitable for analysis were examined in each biopsy (range 20–36). The extent of tubular (dilatation, basement membrane disruption, cell destruction) and interstitial (cellular infiltration, fibrosis, widening) damage was evaluated in successive fields in the complete cortical and juxtamedullary areas examined with an ocular lense, fitted with a grid as described in previous communications (1).

The number of glomeruli in the cortical region (\( n \text{ glomeruli/mm}^2 \text{ of cortex} \)) was evaluated in each biopsy. Glomerular volume was calculated, as previously described (26), using the following equation: \( V_g = \frac{(4/3) \pi \times A_g^{3/2}}{\eta} \), where \( V_g \) is the glomerular volume, \( A_g \) is the glomerular area; \( \beta \) is 1.38 (the shape coefficient for spheres), and \( \kappa \) is 1.1 (the size distribution coefficient). At least 20 glomeruli were examined in each biopsy. Glomerular collapse was identified when the glomerular tuft occupied <50% of the Bowman’s space.

Avidin-biotin-peroxidase methodology was used to identify lymphocytes (CD3 positive cells) and macrophages (F4/80 positive cells). Details of the immunostaining techniques in our laboratories have been described previously (15). Positive cells detected with the immune stainings were studied separately in the glomeruli (positive cells/gcs) and in tubulointerstitial areas (positive cells/mm²).

All histological and immunohistological studies were done without previous knowledge of the group under scrutiny. An Olympus BX51 System Microscope and DP70 microscope digital camera with Sigma Pro (Leesburgh, VA) software were used for computer-assisted image analysis.

**Antisera.** Anti-CD3 monoclonal antibodies (rat anti-human CD3, clone CD3–12 with cross-reactivity with mouse CD3, Serotec Raleigh, NC) and anti-mouse F4/80 monoclonal antibodies (clone C1:A3–1, Fitzgerald Industries, Concord, MA) were used to identify lymphocytes and macrophages, respectively. Dilution used for both antibodies was 1:30. Secondary antibodies were peroxidase-conjugated secondary F(ab’2) rabbit anti-rat IgG (Fitzgerald Industries, Concord, MA). Nonrelevant antibodies were used for negative control studies.

**Statistical analysis.** ANOVA was used in statistical analysis of the data. Correlations were examined with Pearson’s tests. Commercially available programs (GraphPad Instat and GraphPad Prism, GraphPad Software, San Diego, CA) were used for statistical analyses of the data and preparation of graphs. Data are shown as means ± SD. Two-tailed \( P \) values of <0.05 were considered significant.

**RESULTS**

**General data.** No significant difference was observed in body weight between the SOD2\(^{+/−}\) and wild-type mice at either 6 mo or 2 yr of age. Likewise, no significant difference was noted in systolic arterial pressure between the two groups.

![Fig. 1. Systolic blood pressure (A), plasma malondialdehyde (MDA, B), and plasma urea (C) in 2-yr-old superoxide dismutase (SOD2)-deficient (+/−) mice (shaded bars) and wild-type (SOD2\(^{++}\)) mice (open bars). Values are means ± SD. * \( P < 0.05 \).](http://jap.physiology.org/content/102/1/256)
at 6 mo of age (wild-type = 100 ± 7 mmHg; SOD2+/− = 112 ± 17; P = not significant). However, arterial pressure was mildly but significantly higher in the SOD2+/− group than in the wild-type group at 2 yr of age. This was accompanied by a significantly higher serum urea and plasma malondialdehyde assay concentrations in the SOD2-deficient group (Fig. 1).

Heart weights (g) at 2 yr of age were not significantly different in the wild-type and SOD2-deficient mice (SOD2+/− = 0.155 ± 0.18 g; SOD2+/− = 0.173 ± 0.036 g). Similarly, there were no significant differences in kidney weights (right kidney SOD2+/− = 0.263 ± 0.007 g; SOD2+/− = 0.268 ± 0.039 g). There were no significant differences between right and left kidneys (data not shown).

Response to high-salt diet. Baseline arterial blood pressure obtained at 6 mo of age in animals consuming regular diet was similar among the SOD2-deficient and the wild-type animals. Consumption of the high-salt diet resulted in a gradual rise in arterial pressure in the SOD2+/− group but had no effect on blood pressure in the wild-type group. This observation points to the presence of salt sensitivity among the SOD2-deficient mice. Data are illustrated in Fig. 2.

Renal histology and immune cell infiltration data. Renal histology was essentially normal and similar in SOD2-deficient and wild-type mice at 6 mo of age (glomerulosclerosis index score = 0.6 ± 1.0 in the wild-type and 0.7 ± 1.2 in the SOD2-deficient mice; tubulointerstitial damage score = 1.3 ± 1.1% in the wild-type and 2.3 ± 2.30 in the SOD2-deficient mice). In contrast, as described below, at 2 yr of age glomerulosclerosis index and tubulointerstitial damage score were significantly greater in the SOD2+/− than in the wild-type mice (Figs. 3, A and B, and 4, A and B).

At 2 yr of age, the mean number of glomeruli in the renal cortex was lower in the SOD2-deficient (SOD2+/−) group (6.6 ± 1.12 glomeruli/mm²) than in the wild-type (SOD2+/+) group (7.2 ± 1.5 glomeruli/mm²). However, the difference did not reach statistical significance. In general, glomeruli were well preserved. Mesangial expansion was frequently observed in both the SOD2+/− and in the SOD2+/− mice. Glomerular collapse was observed in 13.7 ± 5.31% of the glomeruli in the SOD2+/− and in 20.5 ± 14.3% of the glomeruli in the SOD2+/− mice (P = not significant). The mean glomerular volume (μm³ × 10⁶) was higher in the SOD2+/− mice than in the wild-type (SOD2+/− mice = 1.12 ± 1.21; wild-type mice = 0.44 ± 0.038). However, due to the large glomerular size variability in the SOD2+/− group, the difference did not reach statistical significance.

The infiltration of lymphocytes and macrophages in glomeruli was very rare and did not differ among the wild-type and the SOD2+/− mice (<1 positive cell/gcs in all animals). At 6 mo of age, infiltration of lymphocytes (CD3 positive cells) and macrophages (F4/80 positive cells) in tubulo-interstitial areas was scarce and comparable in the wild-type (CD3 positive cells/mm² = 25.5 ± 3.38; F4/80 positive cells/mm² = 13.0 ± 1.73) and in the SOD2-deficient mice (CD3 positive cells/mm² = 20.6 ± 3.19; F4/80 positive cells/mm² = 18.0 ± 2.00).
However, at 2 yr of age, the SOD2+/− mice had a mild but significant increase in the immune cell infiltration in tubulo-interstitial areas. These findings are shown in Fig. 5, A and B.

Data on the immune cell infiltration in animals fed a high-salt diet are shown in Fig. 6. Infiltration of both lymphocytes (CD3+ cells) and macrophages (F4/80+ cells) in the tubulo-interstitial areas was more intense in the salt-loaded SOD2+/− mice than in the corresponding wild-type group (Fig. 6).

A significant direct correlation was found between arterial blood pressure and the number of infiltrating macrophages in tubulo-interstitial areas among mice included in the dietary salt experiments (Fig. 7).

Fig. 4. A: renal histology in SOD2+/− mice showing focal areas of tubulointerstitial infiltration and tubular dilatation, and two glomeruli showing focal areas of mesangial expansion. B: renal histology in the wild-type (SOD2+/+) mice showed no abnormality.

Fig. 5. A: tubulointerstitial lymphocyte (CD3 positive cells) and macrophage (F4/80 positive cells) infiltration is significantly increased in SOD2+/− mice (shaded bars) compared with the wild-type mice (open bars). B and C: representative microphotographs for wild-type SOD+/+ (B) and SOD2+/− (C). Data are means ± SD. *P < 0.05; **P < 0.01.
DISCUSSION

Earlier studies have demonstrated that partial SOD2 deficiency results in mitochondrial damage by reactive oxygen species resembling cellular senescence (9). Consistent with these observations, mice with partial-SOD2 deficiency (SOD2+/−) showed several features that are reminiscent of the renal and blood pressure modifications associated with aging in humans. For instance, compared with the control animals, the SOD2+/− group showed increased glomerulosclerosis and tubulointerstitial damage (cellular infiltration and tubular dilatation) as well as a mild reduction of renal function at 2 yr of age. These changes are consistent with those described in the humans with advanced age (5, 7, 10, 16, 27). It should be noted that these changes, although significant, were, nevertheless, mild and did not represent advanced renal disease. Similar changes are frequently found in normal humans after the sixth decade of life. For instance, according to a recent study, glomerulosclerosis was found in ~8% of the glomeruli, and tubular atrophy and interstitial fibrosis were observed in 2–8% of the tubulointerstitial areas in otherwise normal individuals aged 71–88 yr (11).

Arterial blood pressure increased with aging in SOD2-deficient but not in the control mice. In addition, blood pressure rose significantly with a high-salt diet in the SOD2-deficient but not in the wild-type mice. The rise in arterial blood pressure in response to increased dietary salt intake in SOD2-deficient mice is indicative of a salt-sensitive state. These observations are reminiscent of the age-associated rise in blood pressure and salt sensitivity seen in ~60% of the elderly individuals (13, 30). The age-associated rise in blood pressure and salt sensitivity in SOD2-deficient mice was accompanied by oxidative stress, as evidenced by elevation of plasma plasma malondialdehyde assay in these animals (Fig. 1B).

The SOD2+/− mice showed a significant infiltration of lymphocytes and macrophages in the tubulointerstitial areas of the kidney. Since oxidative stress is inextricably associated with tubulointerstitial infiltration of immune cells, accumulation of inflammatory cells in this model is likely due to the SOD2 deficiency-induced oxidative stress in the kidney. In turn, the tubulointerstitial inflammatory infiltrate and the associated oxidative stress facilitate sodium retention and salt-driven rise in arterial blood pressure, as shown in several other models of hypertension (reviewed in Refs. 24, 31).

The findings of the present study emphasize the importance of mitochondrial antioxidant system in regulation of blood pressure in response to changes in dietary salt intake. As with the SOD2+/− mice, mice lacking cytosolic SOD (SOD1−/−) and extracellular SOD (SOD3−/−) consuming regular diet do not exhibit spontaneous hypertension at young age (6, 8). Nonetheless, SOD1+/− mice exhibit impaired arterial vasorelaxation (6), and SOD3 knockout mice exhibit exaggerated hypertensive response when subjected to unilateral renal artery stenosis or angiotensin II infusion (8). It should be emphasized that the animals employed in the latter studies were quite young and were fed a regular diet. Thus the effects of aging and high-salt diet on blood pressure in SOD1- and SOD3-deficient mice were not explored in those studies and remain to be investigated.

In summary, the present study demonstrated several morphological features of the aging kidney in mice with partial mitochondrial SOD deficiency. These abnormalities were associated with oxidative stress, renal tubulointerstitial immune cell infiltration, as well as propensity to hypertension with aging and high dietary salt intake. The association of salt sensitivity with oxidative stress and immune cell infiltration of the kidney found in the heterozygote SOD2-deficient mice is consistent with the pattern commonly seen with senescence and various forms of salt-sensitive hypertension.

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