SOD1-Deficiency Causes Salt-Sensitivity and Aggravates Hypertension in Hydronephrosis

Short title: Oxidative stress, Hydronephrosis & Hypertension

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Abstract

Background: Hydronephrosis causes renal dysfunction and salt-sensitive hypertension, which is associated with NO-deficiency and abnormal tubuloglomerular feedback (TGF) response. We investigated the role of oxidative stress for salt-sensitivity and for hypertension in hydronephrosis.

Methods: Hydronephrosis was induced in SOD1-transgenic (SOD1-tg), SOD1-deficient (SOD1-ko) and wild-type mice and in rats. In mice, telemetric measurements were performed during normal (0.7% NaCl) and high sodium (4% NaCl) diets and with chronic Tempol supplementation. 8-iso-prostaglandin-F$_2$α (F2-IsoPs) and protein excretion profiles and histology were investigated. The acute effects of Tempol on blood pressure and TGF were studied in rats.

Results: In hydronephrosis, wild-type mice developed salt-sensitive hypertension (114±1 to 120±2 mmHg) which was augmented in SOD1-ko (125±3 to 135±4 mmHg), but abolished in SOD1-tg (109±3 to 108±3 mmHg). SOD1-ko controls displayed salt-sensitive blood pressure (108±1 to 115±2 mmHg), which was not found in wild-types or SOD1-tg. Chronic Tempol treatment reduced blood pressure in SOD1-ko controls (-7 mmHg) and in hydronephrotic wild-types (-8 mmHg) and SOD1-ko mice (-16 mmHg), but had no effect on blood pressure in wild-type or SOD1-tg controls. SOD1-ko controls and hydronephrotic wild-type and SOD1-ko mice exhibited increased fluid excretion associated with increased F2-IsoPs and protein excretion. The renal histopathological changes found in hydronephrotic wild-types were augmented in SOD1-ko and diminished in SOD-tg mice. Tempol attenuated blood pressure and normalized TGF response in hydronephrosis (ΔP$_{SF}$: 15.2±1.2 to 9.1±0.6 mmHg, TP: 14.3±0.8 to 19.7±1.4 nl/min).

Conclusion: Oxidative stress due to SOD1-deficiency causes salt-sensitivity and plays a pivotal role for the development of hypertension in hydronephrosis. Increased superoxide formation may enhance TGF response and thereby contribute to hypertension.
Introduction

Hydronephrosis, due to obstruction at the level of the pelvic-ureteric junction is a common condition in children, with an incidence in newborns of approximately 1%. Chronic partial unilateral ureteral obstruction causes renal injury and salt-sensitive hypertension in both rats (7) and mice (8). Reduced nitric oxide (NO) availability in the diseased kidney, increased afferent arteriolar resistance and resetting of the tubuloglomerular feedback (TGF)-mechanism, have important roles in the development of hypertension (4, 5). The cause of NO-deficiency in hydronephrosis is not clear, but oxidative stress in the diseased kidney has been suggested (5).

There is a close relationship between renal oxidative stress and development and maintenance of hypertension (51). Reactive oxygen species (ROS) are constantly formed during cellular metabolism, and under normal conditions ROS play a critical role in the signalling mechanisms that control cellular function. Oxidative stress implies an imbalance between the production and degradation of oxidants, in favor for the oxidants (3, 52) and has been demonstrated during high sodium intake and in the pathogenesis of renal, cardiovascular and metabolic diseases (18, 19, 45, 46). The primary ROS produced is superoxide (O$_2^-$) which is predominantly formed by NADPH oxidases. This reactive anion can be metabolized by superoxide dismutase (SOD) to hydrogen peroxide (H$_2$O$_2$), or by interaction with NO to form peroxynitrite (ONOO$^-$) (37).

The three SOD-isoforms [(CuZnSOD; SOD1), (MnSOD; SOD2) and (ECSOD; SOD3)] are all expressed within the vessel wall. (37). Complete SOD2-deficiency results in neonatal lethality (26), but heterozygous SOD2 mice develop mild salt-sensitive hypertension and renal senescence with age (37). SOD3-deficient mice have been demonstrated to have increased oxidative stress, impaired NO bioavailability, an increased renal vascular resistance and display normal (16) or elevated blood pressure (47) during normal sodium intake. This isoform is also critical in preventing Angiotensin II induced hypertension and endothelial dysfunction (15, 16, 47).
It has been suggested that SOD1, which is the predominant isoform in blood vessels (13), is important for the release of NO from the endothelium (33). SOD1-deficient mice are not hypertensive during normal sodium intake (13), but there is lack of information regarding the role of SOD1 in blood pressure regulation during high sodium intake.

Enhanced production of $O_2^-$ can influence renal hemodynamic and blood pressure directly or indirectly via a reduction in NO bioavailability (51). Administration of Tempol (SOD-mimetic) has been demonstrated to attenuate oxidative stress and blood pressure in experimental models for hypertension (1, 17, 20, 34, 38, 48). Furthermore, treatment modalities that increase NO formation (30) or inhibit oxidative stress (41) are beneficial to the progression of cellular and molecular parameters of tubulointerstitial fibrosis caused by unilateral ureteral obstruction; whereas, NO-deficiency increases renal damage (21).

In vivo studies of single nephron function and in vitro studies with the double-perfused juxtaglomerular apparatus preparation have shown extensive interaction between $O_2^-$ and NO in macula densa (MD) to regulate afferent arteriolar tone, mediated by the TGF (50). Enhanced TGF responsiveness, due to loss of nNOS-derived NO, has been demonstrated in spontaneously hypertensive rats (SHR) (49), Milan hypertensive strains (MHS) of rats (43) and in hydronephrotic rats (4), and is thought to be an important contributor of hypertension.

In the present study the role of oxidative stress for salt-sensitivity and for the development of hypertension in unilateral hydronephrosis was investigated. Based on earlier observations(4, 5, 7), it was hypothesized that renal NO-deficiency and subsequent hypertension was a consequence of increased oxidative stress in the diseased kidney. Blood pressure, renal excretion of fluid, electrolytes and 8-iso-prostaglandin $F_{2\alpha}$ (F2-IsoPs) were measured continuously, and the renal histology was examined in SOD1-transgenic, knock-out and wild-type mice. The acute effects of Tempol on blood pressure and TGF responsiveness were evaluated. Due to experimental difficulties associated with TGF measurements in hydronephrotic mice we used rats to investigate the role of oxidative stress for the resetting of the abnormal TGF-response.
**Methods**

In *series I*, the experiments were carried out on homozygous littermates from breeding pairs of SOD1-transgenic (*Tg(SOD1)3Cje/J; SOD1-tg*) or knock-out mice (*Sod1tm1Leb; SOD1-ko*) from The Jackson Laboratory, Maine, USA. C57BL/6J mice from the breeding colony served as wild-type controls. In *series II*, male Sprague-Dawley rats (Møllegaard, Denmark) were used.

**Creation of partial unilateral ureteral obstruction**

A partial unilateral ureteral obstruction (PUUO) was created in 3-week-old mice and rats to induce hydronephrosis, as described previously (7, 8). In short, anaesthesia with spontaneous inhalation of isoflurane (Forene®, Abbot Scandinavia AB, Kista, Sweden) was used and the abdomen was opened sterile through a midline incision and the left ureter was isolated and embedded in the underlying psoas muscle. Sham operations in control animals were performed in the same way, but without dissecting the ureter. All animals were then left to grow with free access to tap water and standardized diet (*described below*) and the experiments were performed 8-12 weeks (mice) or 5-8 weeks (rats) later.

**Experimental Protocols**

In series I, a standardized normal sodium diet (0.7% NaCl, SD389-R36, Lactamin, Kimstad, Sweden), was followed by a period with high sodium diet (4% NaCl, SD312-R36, Lactamin, Kimstad, Sweden). The adult mice given a high sodium diet were allowed to equilibrate for 10 days on the new diet before the measurements were commenced (i.e. high sodium diet, with or without chronic Tempol (4-Hydroxi-Tempo, Sigma-Aldrich) supplementation. In series II, rats were only given a normal sodium diet and the acute effects of Tempol on blood pressure and TGF were investigated. In both series I and II the experiments were carried out in sham-operated controls and in hydronephrotic animals.
**Series I**

**Telemetric blood pressure measurements**

The telemetric device (PA-C10, DSI™, St Paul, MN, USA) was implanted in adult mice (i.e. 8-12 weeks following PUUO). After surgery, all animals were allowed to recover for at least 10 days before any measurements commenced. Telemetric measurements of mean arterial blood pressure was conducted continuously for at least 48 hours on both normal and high salt diets, as previously described (8). The effect of chronic Tempol was investigated during high sodium intake. Blood pressure was recorded during a control period (72 hours) and then continuously for 8 days with Tempol supplementation (2 mM) in the drinking water.

**Renal excretion measurements**

Immediately following the telemetric measurements on normal sodium diet, the mice were placed in metabolism cages, for 24-hours, with food and water given ad libitum to study renal excretion of electrolytes, fluid, F2-IsoPs and proteins. Water consumption and diuresis were measured gravimetrically. Urine osmolality was determined by using an osmometer (Fiske 210 Micro-Sample Osmometer, Norwood, MA, USA) and sodium and potassium concentrations were determined by flame photometry (FLM3, Radiometer, Copenhagen, Denmark).

**8-Isoprostane and Protein analysis**

Samples of fresh urine, collected from the renal excretion measurements, were stored at –70°C until analyzed. The urinary content of F2-IsoPs was analyzed by competitive enzyme-linked immunoassay (Bioxytech 8-Isoprostone Assay, OxisResearch, Portland, USA) (32). Measurement of the rate of F2-IsoPs excretion is advantageous compared with plasma analysis due to minimal ex-vivo formation and 24-hours collection provides an integrated F2-IsoPs production with time.
Urinary protein content was determined by the colorimetric method of DC Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA). Plates were read from the bottom using a microplate reader (Safire II, Tecan Austria GmbH, Grödig, Austria) (Absorbance at 750 nm).

**Histology**

The kidneys were explanted and to determine the degree of hydronephrosis, the hydronephrotic ratio (HNR) was calculated as the residual urine weight divided by the renal parenchymal weight (8). Embedded tissue blocks were cut into 5-µm thick sections and stained with Haematoxylin and Eosin (HE), Periodic Acid-Schiff (PAS) stain and Picro-Sirius stain for a blind histological evaluation. The renal cortex, medulla and the papilla were investigated for fibrosis, inflammation (i.e. infiltration of plasma cells and lymphocytes), tubular changes (i.e. hyaline material and atrophy) and glomerular changes (i.e. sclerosis, mesangial matrix increase and shrunken glomeruli). The tissues evaluated were given a score of 0-4, depending on the severity of change (0=no changes, 1=detectable changes, 2=mild, 3=moderate and 4=severe changes). The lowest score was given if the renal histoarchitecture was normal, with no changes in any of the investigated parameters. The highest score represented major distortion of the normal histoarchitecture in both cortex and medulla.

**Series II**

**Effects of Tempol on blood pressure and TGF characteristics**

The rats were anaesthetised with thiobutabarbitral (100mg/kg i.p.), (Inactin®, Sigma-Aldricht), and prepared for blood pressure and renal micropuncture, as described earlier (4). Throughout the experiments, rats received isotonic saline intravenously (5ml/kg/h), and after surgery a 45-mins equilibration period was allowed before any measurements were conducted. Blood pressure was measured in a 30-mins baseline period (B) and during four consecutive experimental 30-mins periods (E1-E4) with infusion of Tempol (200µmol/kg/h i.v.) (4-Hydroxi-Tempo, Sigma-
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Aldrich) or saline. The TGF characteristics were determined in the hydronephrotic kidney by stop-flow technique, before and at least 20-mins after continued administration of Tempol.

Stop-flow measurements: Randomly chosen early proximal tubular segments on the surface were punctured with a sharpened glass pipette (OD 3-5 μm) filled with lissamine green-stained 1M NaCl solution. The pipette was connected to a servo-nulling pressure system (World Precision Instruments, New Haven, CT, USA) to determine the proximal tubular free-flow pressure (Pf). A second pipette (OD 7-9 μm), filled with artificial ultrafiltrate (in mM: 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 4 NaHCO₃, 7 urea, and 2 g/l Lissamine green, pH 7.4) and connected to a microperfusion pump (Hampel, Frankfurt, Germany), was inserted in the last accessible segment of the proximal tubule. A solid wax block was placed in between with a third pipette (OD 7-9 μm). The proximal tubular stop-flow pressure (Psf) upstream to the block was determined at different perfusion rates (0-40 nl/min) in the loop of Henle. The maximal change in stop-flow pressure (ΔPsf), was used to indicate the TGF reactivity and the tubular flow rate, eliciting half-maximal ΔPsf, [i.e. the turning point (TP)], indicated the TGF sensitivity. The TGF-response curves with normalized data were created using a non-linear least-squares curve fitting program (2).

Calculations and statistics

Values are presented as mean ± SEM. Single comparisons between normally distributed parameters were tested for significance with Student’s paired or unpaired t-test. For multiple comparisons, analysis of variance (ANOVA) followed by the Fisher’s post-hoc test were used. For the stop-flow pressure measurements, multiple groups were compared by two-way ANOVA. The Bonferroni post-test for paired multiple comparisons, was used to allow for more than one comparison with the same variable. This states a significance level of P/M, where M is the number of comparisons to be made. Scored data for the histological evaluation was analysed by
the Kruskal-Wallis test followed by the Mann-Whitney-U-test. Statistical significance was defined as P<0.05.

Ethics

The experiments were approved by The Uppsala Ethical Committee for Animal Experiments.
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Results

All animals used in this study were in good condition, and at the beginning of the experiments there were no differences in body weight between the different groups in series I or in series II. In series I, an equal number of male and female mice were used for all investigations. The age of the mice when the experiments were started (i.e. implantation of telemetric device) did not differ between the genotypes (SOD1-tg: 13±1; SOD1-ko: 13±1; wild-type: 13±1 weeks).

In series II, only male rats were used with an age between 8-11 weeks.

Series I:

Telemetric blood pressure measurements – Effects of different salt diets

The results from the blood pressure measurements during different sodium diets are shown in Figure 1, and as data supplement.

Wild-type: Hydronephrotic wild-type mice (n=9) developed hypertension that was salt-sensitive. The blood pressure increased from 114±1 mmHg on normal salt diet to 120±2 mmHg on high salt diet, compared with 103±2 to 105±1 in controls (n=9).

SOD1-tg: The hydronephrotic SOD1-tg mice (109±3 mmHg, n=8) and the SOD1-tg controls (106±1mmHg, n=8) were normotensive and did not display any salt-sensitivity.

SOD1-ko: In the hydronephrotic SOD1-ko mice (n=9) the hypertension aggravated compared with the hydronephrotic wild-type mice. Furthermore, the salt-sensitivity (i.e. change in mean arterial pressure when given high sodium diet) was also augmented compared with the hydronephrotic wild-type mice (P=0.046). The blood pressure increased from 125±3 mmHg on normal salt diet to 135±4 mmHg on high salt diet, compared with 108±1 to 115±2 in SOD1-ko controls (n=9). SOD1-ko controls displayed salt-sensitive blood pressure (+7 mmHg), and on a high sodium diet SOD1-ko controls had a higher blood pressure than controls of both wild-type and SOD1-tg mice.
The mean locomotor activity levels during the different sodium periods are shown in Figure 2. No differences were found between the diets and groups of the same genotype. The SOD1-tg controls had a higher locomotor activity compared with wild-type controls during normal sodium intake, but not significant during high sodium intake (P=0.06). Furthermore, we did not find any gender differences in basal blood pressure, salt-sensitivity or in locomotor activity.

**Telemetric blood pressure measurements – Effects of Tempol**

The results from the blood pressure measurements during chronic Tempol supplementation are shown in Figure 3. Tempol lowered blood pressure over time in hydronephrotic wild-types, SOD1-ko controls and in hydronephrotic SOD1-ko mice, but had no effect in wild-type controls. The blood pressure level during Tempol treatment (i.e. 8 days mean) was reduced by 8 mmHg in hydronephrotic wild-types, 7 mmHg in SOD1-ko controls and by 16 mmHg in hydronephrotic SOD1-ko mice. Similar to wild-type controls, Tempol had no effect on blood pressure in SOD1-tg controls (data not shown).

**Renal excretion measurements**

Renal excretion data are summarized in Table 1. Urine excretion rate was lower in the SOD1-tg group and higher in the SOD1-ko group compared with their corresponding controls in the wild-type group. For all genotypes, the diuresis of the hydronephrotic mice tended to be higher than their corresponding controls. The largest difference, between controls and hydronephrotic animals, were found in the SOD1-ko group (26 µl/24h/g bw) and the smallest difference in the SOD1-tg group (8 µl/24h/g bw). Urine osmolality was reduced in the SOD1-ko group compared with corresponding controls of SOD1-tg and wild-type mice. In the hydronephrotic wild-type
and SOD1-ko group the animals tended to have a reduced concentrating ability compared with their corresponding controls. No clear differences were found in the electrolyte excretion.

**Isoprostanes**

Urinary excretion of F2-IsoPs is shown in Figure 4a. Hydronephrotic wild-type mice displayed increased excretion of F2-IsoPs, which was even further elevated in the hydronephrotic SOD1-ko animals, compared with their corresponding controls. Furthermore, the SOD1-ko controls had higher excretion of F2-IsoPs than the controls of wild-type and SOD1-tg. No differences were found between the groups of SOD1-tg mice.

**Proteins**

Urinary protein excretion is shown in Figure 4b. SOD1-ko controls had greater protein excretion than the SOD1-tg mice. No significant differences were found between hydronephrotic wild-types or SOD1-tg mice and their corresponding controls. Hydronephrotic SOD1-ko mice had higher protein excretion than that of the hydronephrotic wild-types and SOD1-tg mice, and also a tendency (P=0.10) for higher excretion compared with the SOD1-ko controls.

**Histology**

The results from the histological evaluation of the kidneys are summarized in Table 2. All the hydronephrotic kidneys from the wild-type, SOD1-tg and the SOD1-ko mice displayed variable degrees of dilatation of the pelvic area, with flattening of the renal papilla. There were no differences in the HNR between the three genotypes (approx. 0.70). Representative photomicrographs of the renal pathological changes are demonstrated in Figure 5. The hydronephrotic kidneys exhibited areas with subepithelial fibrosis, infiltration of inflammatory cells (i.e. plasma cells and lymphocytes), predominantly localized to the medulla and pelvic
region, and glomerular changes (i.e. sclerosis, mesangial matrix increase and collapsed glomeruli). The SOD1-ko mice displayed the most severe changes among all the parameters investigated, whereas the smallest amounts of changes were observed in the SOD1-tg mice. Fibrotic, inflammatory and glomerular changes were also identified in the contralateral kidneys of the hydronephrotic mice. This was associated with a compensatory contralateral hypertrophy in all genotypes. The sham operated controls of all genotypes displayed normal histoarchitecture. No histopathological changes were found in the SOD1-tg controls, whereas in the SOD1-ko controls fibrotic, inflammatory and glomerular changes were observed.

**Series II:**

**Effect of acute Tempol administration on the blood pressure**

The effects of Tempol on the blood pressure in hydronephrotic and control rats are shown in Figure 6. The blood pressure was higher in the hydronephrotic animals during the baseline period and Tempol produced a decrease in blood pressure that was more pronounced in the hydronephrotic animals than in the controls. There was no difference in blood pressure response between the groups that only received saline throughout the experimental period.

**Effect of acute Tempol administration on the TGF characteristics**

The influence of Tempol on the TGF characteristics in control and hydronephrotic kidneys is shown in Table 3 and in Figure 7.

In Figure 7 (panel A), $P_{SF}$ at different perfusion rates are averaged, and the corresponding values are shown during control conditions and following Tempol infusion. Between nephrons there are variations in TP, so that the mean response curve has a different slope compared with each individual response curve. Therefore, to better visualize the response curve for each individual nephron, normalized data are presented in Figure 7 (panel B). The representations in
Figure 7 (panel A and B) are similar in all other aspects except for the width of the perfusion rate interval between which the full response occurs.

The $P_T$, but not $P_{SF}$, was slightly higher in the hydronephrotic group during control conditions, however after Tempol administration no differences were found between the groups. During control conditions, the reactivity of the TGF response, as indicated by the $\Delta P_{SF}$, was greater in the hydronephrotic group (15.2±1.2 mmHg) than in control rats (9.2±0.7 mmHg). Furthermore, the flow-rate eliciting a half-maximal $P_{SF}$ response (i.e. $T_P$) was lower in the hydronephrotic animals (14.3±0.8 nl/min) than in the controls (18.7±1.1 nl/min), indicating a higher sensitivity of the TGF response in hydronephrosis.

Administration of Tempol caused attenuation of the TGF response (decreased $\Delta P_{SF}$ and increased $T_P$) in the hydronephrotic rats, as shown by the rightward shift in Figures 7. However, in the control animals no significant changes were observed. After administration of Tempol there were no longer any differences in the TGF response between the controls and the hydronephrotic animals.
Discussion

We recently demonstrated that hydronephrotic animals with hypertension have reduced NO availability in the obstructed kidney associated with abnormal afferent arteriolar and TGF responsiveness (4, 5). The present study suggests that the development of salt-sensitive hypertension and renal injuries in unilateral hydronephrosis is causally related to oxidative stress (via $O_2^-$).

There is a close relationship between oxidative stress, NO-deficiency and development of hypertension (51). $O_2^-$ is the main ROS in the vasculature and is metabolized by SOD or by scavenging of NO. The three SOD-isoforms are all expressed within the vessel wall, however, SOD1 is the predominant isoform in blood vessels (13). Studies have shown that SOD1-deficient mice on a normal sodium diet, have an increased production of $O_2^-$ and impaired endothelial function in both large vessels and in microvessels, but no hypertension at young age (13). Furthermore, the impaired endothelial function can be restored by treating with the SOD mimetic, Tempol (12). Reduced expression of SOD1 causes a greater increase in Angiotensin II induced vascular $O_2^-$ levels and endothelial dysfunction. In contrast, overexpression of SOD1 prevents the Angiotensin II induced response very effectively (11). It has been demonstrated that high sodium intake can increase oxidative stress in the vasculature and in the kidney (14), accelerate renal and cardiovascular pathological changes and lead to hypertension (6). There is little known regarding the influence of high sodium diet in SOD1-ko mice. Measuring blood pressure with radiotelemetry, in this study, has revealed that SOD1-ko control mice are normotensive during normal sodium intake, but become hypertensive when given a high sodium diet. The wild-type controls and the SOD1-tg controls did not display any significant salt-sensitivity. This finding cannot be explained by changes in the locomotor activity as no differences were found between the diets or groups of the same genotype. As high sodium intake is associated with oxidative stress (14) these findings emphasize the importance of SOD1 in response to changes in dietary salt intake. In the transgenic mice increased formation of $O_2^-$ can
Easily be metabolized via SOD, whereas in the knock-outs, lack of SOD1 may reduce NO bioavailability (via peroxynitrite formation) with subsequent hypertension. In the present study peroxynitrite was not measured, however elevated levels of peroxynitrite and impaired NO mediated vasodilation has been demonstrated in SOD1-ko mice (10).

Increased ROS formation has been demonstrated to contribute to the progression of renal disease in unilateral ureteral obstruction. Administration of antioxidants (29, 35) or AT1-receptor blocker (41, 44) ameliorate renal fibrosis and oxidative stress, preserve renal function in hydronephrotic animals and supplementation with NO-substrate attenuate hypertension (4). The histological evaluation in the present study showed subepithelial fibrosis, inflammatory cells and glomerular changes in hydronephrotic wild-type mice, which were augmented in hydronephrotic kidneys lacking SOD1 and diminished in those overexpressing the enzyme. This finding cannot be explained by different degrees of hydronephrosis since the hydronephrotic ratio was similar in all genotypes. The histopathological differences may be more readily explained by different SOD1 expression. However, blood pressure per se could also contribute to the renal injuries in the hypertensive animals. SOD1-deficiency also increased the degree of histopathological changes in the control mice.

The observed renal injuries in SOD1-deficient mice were associated with increased protein excretion for both control and hydronephrotic groups. These findings support earlier reports of an association between oxidative stress and renal histopathological changes in kidneys with ureteral obstruction (29, 35, 41, 44). Furthermore this study suggests that oxidative stress (via $O_2^-$) triggers and/or exaggerates fibrosis and inflammation in hydronephrotic mice.

Pharmacological inhibition of SOD exacerbates, whereas administration of SOD-mimetic (Tempol) ameliorates oxidative stress and hypertension in experimental models of hypertension (22, 51). The findings of the present study demonstrate a causal link between oxidative stress and the development of hypertension in hydronephrosis. In mice overexpressing SOD1 the hypertension as well as salt-sensitivity was completely abolished, whereas those lacking SOD1
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the salt-sensitive hypertension was exaggerated. The implication of oxidative stress during high sodium intake is further supported by the findings that chronic Tempol supplementation reduced blood pressure in SOD1-ko control, hydronephrotic wild-type and SOD1-ko mice, but had no effect in the wild-type or SOD1-tg control mice.

Measurement of F2-IsoPs, generated as a result of the free radical-mediated peroxidation of arachidonic acid F2-isoprostanes, has emerged as one of the most reliable approaches to assess oxidative stress in vivo (28, 31). F2-IsoPs is not only a marker of oxidative stress, but is also a vasoconstrictor and F2-IsoPs receptors have been located in renal arterial smooth muscle cells (42). In the present study the hypothesis of a role of oxidative stress in development of hypertension in hydronephrosis was supported by the F2-IsoPs analysis. The SOD1-ko controls and hydronephrotic wild-type mice displayed increased excretion of F2-IsoPs compared with the wild-type controls, and in the SOD1-ko hydronephrotic mice the F2-IsoPs levels were even further elevated. It has been demonstrated that SOD1-ko mice have reduced total SOD and increased $O_2^-$ production in the vasculature (13). It is likely that the excessive oxidative stress and exaggerated hypertension found in hydronephrotic SOD1-ko mice is due to renal oxidative stress in the disease kidney. Furthermore, increased $O_2^-$ production and decreased SOD activity has been demonstrated in the diseased kidney of Wistar Kyoto rats with ureteral obstruction (25), and in kidneys of Dahl salt-sensitive rats (27). We believe that in our model for hydronephrosis, oxidative stress is primarily caused by increased renal production of ROS (since renal disease is associated with increased ROS), however we cannot exclude a reduction in antioxidant systems. From our study it is evident that the capacity of the antioxidant systems is insufficient to eliminate the produced ROS.

In previous studies we have shown that animals with hydronephrosis have increased diuresis and impaired renal concentrating ability (4, 8). This is probably caused by pressure diuresis, reduced mass of the renal medulla (4, 8) and down-regulation of aquaporin (23) and sodium transporters (40) in the obstructed kidney. The present study supports earlier findings in
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Hydronephrotic wild-types and do also show that the impaired renal function is worsened in hydronephrotic mice lacking SOD1. This may be a consequence of higher blood pressure in this genotype, causing increased fluid and electrolyte excretion. In the present study renal function in terms of glomerular filtration rate (GFR) was not investigated. In general, ureteral obstruction results in decreased total GFR, which most likely is due to a reduced renal function of the hydronephrotic kidney. Measurements of ipsi- and contralateral excretory function are very difficult to perform, due to large pelvic dead space of the hydronephrotic kidney. Split kidney function is possible to determine after reversal of the obstruction, and has demonstrated a reduced GFR of the ipsilateral kidney (9). However, after reversal of the obstruction the physiological condition will be different compared with the obstructed state.

Chronic Tempol administration normalizes blood pressure in SHR (39) and acute administration (with equivalent doses as used in series I) of Tempol has been shown to attenuate blood pressure in 2K1C hypertensive rats (17). In the present study, Tempol infusion produced a fall in blood pressure in both hydronephrotic (~24%) and in control (~9%) rats. In the rats receiving saline only, the blood pressure was reduced by about 6% in both groups. Taken together, the blood pressure response was much more pronounced in the hydronephrotic animals and therefore suggests a role for $O_2^-$ in perpetuating hypertension. These findings are similar to those observed in 2K1C hypertensive rats.

The TGF contributes to the renal autoregulation, and is modulated by Angiotensin II and NO (36). Since the MD cells express both nNOS and NADPH oxidases it has been suggested that the TGF response may also be regulated by interaction between NO and $O_2^-$. Enhanced TGF responsiveness has been demonstrated in experimental models for hypertension (i.e. SHR, MHS rats, chronic 7-NI treated rats and in hydronephrosis). In SHR, MHS and hydronephrotic rats, reduced NO availability in the MD has been demonstrated (4, 43, 49). The enhanced responsiveness of the TGF in SHR is dependent on increased $O_2^-$ production (50). $O_2^-$ enhances the TGF, directly by constricting afferent arterioles and indirectly by scavenging NO in the MD.
Studies in isolated and perfused afferent arterioles have shown that the NO availability is reduced in SOD1-ko compared to that of SOD1-tg mice. Furthermore, hydronephrotic wild-type mice have a reduced NO production in the partially obstructed kidney (5). In the present study the TGF reactivity and sensitivity were increased in the hydronephrotic kidney. Tempol caused a desensitization of the TGF response (i.e. rightward shift) whereas in the controls no change was observed. This indicates that the reduced renal NO availability in hydronephrosis, as demonstrated previously, may be due to increased O$_2^-$ formation.

**In conclusion**, oxidative stress plays a pivotal role for salt-sensitive hypertension in hydronephrosis. Increased superoxide formation may contribute to the enhanced TGF response and thereby be involved in the development and maintenance of hypertension. Furthermore, the study emphasizes the association between oxidative stress due to SOD1-deficiency and salt-sensitivity.

**Perspectives and Significance**

Oxidative stress has been implicated in various disease states. Emerging evidence from experimental studies suggests that increased ROS formation in the kidney is a key factor in the development and persistence of hypertension. We have previously demonstrated that hydronephrosis, due to chronic ureteral obstruction, is associated with renal NO-deficiency, increased TGF responsiveness and salt-sensitive hypertension. From the present study one could speculate that increased O$_2^-$ levels in hydronephrosis can cause hypertension by enhancing the TGF response, both directly by vasoconstriction of the afferent arterioles and indirectly by scavenging NO in the MD. Furthermore, the study emphasizes a correlation between SOD1-deficiency and salt-sensitivity. During conditions of high O$_2^-$ production (e.g. high sodium intake) the SOD1-isoform has an important role in maintaining arterial blood pressure.

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Disclosures

None
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References


Figure 1.
Mean arterial pressures in conscious wild-type, SOD1-transgenic (SOD1-tg) and SOD1-deficient (SOD1-ko) controls and hydronephrotic mice, treated with normal sodium or high sodium diets. The telemetric measurements were conducted continuously for at least 48 hours. Values are presented as mean ± SEM
* P<0.05
# P<0.05 compared with same group and diet for the SOD1-ko
† P<0.05 compared with same group and diet for the SOD1-tg.

Figure 2.
Mean locomotor activity in conscious wild-type, SOD1-transgenic (SOD1-tg) and SOD1-deficient (SOD1-ko) controls and hydronephrotic mice, on normal sodium or high sodium diets. The telemetric measurements were conducted continuously for at least 48 hours. Values are presented as mean activity counts per hour ± SEM
No significant differences were found between the diets and groups of the same genotype.
* P<0.05 compared with same group and diet for wild-type mice.

Figure 3.
Effects of Tempol supplementation on mean arterial pressures in conscious wild-type and SOD1-deficient (SOD1-ko) controls and hydronephrotic mice on high sodium diet. The telemetric measurements were conducted under control conditions (Baseline; B) for 3 days and then continuously with Tempol in the drinking water for 8 days. Values are presented as mean ± SEM
* P<0.05 compared with baseline within same group and genotype.
Figure 4a.
Urinary excretion of 8-iso-prostaglandin F$_{2\alpha}$ in wild-type, SOD1-transgenic (SOD1-tg) and SOD1-deficient (SOD1-ko) controls and hydronephrotic mice. Values are during 24 hours in metabolism cages. Values are presented as mean ± SEM.
* P<0.05

Figure 4b.
Urinary protein excretion in wild-type, SOD1-transgenic (SOD1-tg) and SOD1-deficient (SOD1-ko) controls and hydronephrotic mice. Values are during 24 hours in metabolism cages. Values are presented as mean ± SEM.
* P<0.05

Figure 5.
Representative photomicrographs of renal tissue from wild-type, SOD1-transgenic (SOD1-tg) and SOD1-deficient (SOD1-ko) controls and hydronephrotic mice. A-F are stained with Picro-Sirius for visualizing interstitial fibrosis, especially collagen. G-L are stained with Haematoxylin & Eosin for visualizing inflammation. Normal histology with absence of fibrosis and inflammation in controls of wild-type (A+G) and SOD1-tg mice (C+I). E demonstrates areas with subepithelial fibrosis (black arrow) and mild glomerular changes with mesangial matrix increase (white arrow) in SOD1-ko control. B+D demonstrate increase of fibrotic tissue in the interstitium (black arrows) and in the glomerulus (white arrow) of hydronephrotic wild-type and SOD1-tg mice. F demonstrates areas with severe cortical (black arrow) and glomerular changes with matrix increase, sclerosis and shrunken glomerulus (white arrows) in hydronephrotic SOD1-ko mouse. K demonstrates infiltration of inflammatory cells (mainly lymphocytes) in the cortex of SOD1-ko control. Moderate (H) and mild (J) chronic inflammatory infiltrate (mainly lymphocytes) in the medulla and pelvic region (white arrows) of hydronephrotic wild-type and
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SOD1-tg mice. L demonstrates areas with severe chronic inflammatory changes (plasma cells and lymphocytes) in the cortical medullary region (white arrows) of hydronephrotic SOD1-ko mouse. Scale bar: 100µm

**Figure 6.**

Effects of Tempol (200µmol/kg/h i.v.) or vehicle (isotonic saline) on blood pressure in anaesthetized hydronephrotic and control rats. B=Baseline period, E1-E4=four consecutive experimental periods (30mins each). Values are presented as mean ± SEM.

* P<0.05 compared with control values of same group

* # P<0.05 compared with values of control animals under similar conditions

**Figure 7.**

*Panel A:* Proximal tubular stop-flow pressure, at different tubular perfusion rates, in controls and hydronephrotic rats (i.e. partially obstructed kidney). Curves show actual data under control conditions and after administration of Tempol.

*Panel B:* Proximal tubular stop-flow pressure, at different tubular perfusion rates, in controls and hydronephrotic rats (i.e. partially obstructed kidney). Curves are results of fitting of normalized data under control conditions and after administration of Tempol.

*P<0.05 compared with baseline values of same group

* #P<0.05 compared with values of control animals under similar conditions.
Table 1. Renal excretion data of wild-type, SOD1-transgenic (SOD1-tg) and SOD1-deficient (SOD1-ko) controls and hydronephrotic mice from metabolism cages

<table>
<thead>
<tr>
<th></th>
<th>wild-type</th>
<th>SOD1-tg</th>
<th>SOD1-ko</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Hydronephrosis</td>
<td>Controls</td>
</tr>
<tr>
<td>Water intake (μl/24 h/g bw)</td>
<td>75±15 86±13</td>
<td>77±14 84±11</td>
<td>117±11&lt;sup&gt;ac&lt;/sup&gt; 132±16&lt;sup&gt;bd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diuresis (μl/24 h/g bw)</td>
<td>76±5   89±7&lt;sup&gt;*&lt;/sup&gt;</td>
<td>51±6&lt;sup&gt;a&lt;/sup&gt; 59±9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>111±16&lt;sup&gt;ac&lt;/sup&gt; 137±9&lt;sup&gt;bd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt; (mM)</td>
<td>86±10  60±6&lt;sup&gt;*&lt;/sup&gt;</td>
<td>108±13 106±9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64±4&lt;sup&gt;ac&lt;/sup&gt; 62±9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt; excretion (μmol/24 h/g bw)</td>
<td>6.9±0.9 5.4±0.7</td>
<td>5.2±0.8 5.5±0.6</td>
<td>6.6±0.9 8.7±0.7&lt;sup&gt;bd&lt;/sup&gt;</td>
</tr>
<tr>
<td>K&lt;sup&gt;+&lt;/sup&gt; (mM)</td>
<td>125±14 96±7&lt;sup&gt;*&lt;/sup&gt;</td>
<td>135±23 129±13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90±13&lt;sup&gt;a&lt;/sup&gt; 69±9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>K&lt;sup&gt;+&lt;/sup&gt; excretion (μmol/24 h/g bw)</td>
<td>10.3±1.3 8.4±0.8</td>
<td>6.5±1.2&lt;sup&gt;a&lt;/sup&gt; 7.0±1.0</td>
<td>8.8±1.3 9.1±1.3</td>
</tr>
<tr>
<td>Osmolality (mM)</td>
<td>1237±109 1043±44&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1253±191 1277±83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>890±111&lt;sup&gt;a&lt;/sup&gt; 757±83&lt;sup&gt;bd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Osmolar excretion (μOsm/24 h/g bw)</td>
<td>96±9 95±6</td>
<td>67±8&lt;sup&gt;a&lt;/sup&gt; 71±8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86±9 105±12&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>n</td>
<td>12 14</td>
<td>8 10</td>
<td>11 11</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM. n=number of animals, bw=body weight

* P<0.05 compared with Controls (same genotype)

<sup>a</sup> P<0.05 compared with wild-type (Controls)

<sup>b</sup> P<0.05 compared with wild-type (Hydronephrosis)

<sup>c</sup> P<0.05 compared with SOD1-tg (Controls)

<sup>d</sup> P<0.05 compared with SOD1-tg (Hydronephrosis)
Table 2. Histology of the kidneys from wild-type, SOD1-transgenic (SOD1-tg) and SOD1-deficient (SOD1-ko) controls and hydronephrotic mice

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Hydronephrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wild-type</td>
<td>SOD1-tg</td>
</tr>
<tr>
<td></td>
<td>n=10</td>
<td>n=7</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>kw / bw (x10⁻³)</td>
<td>5.7 ± 0.3</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>HNR</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Glomerular changes</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>kw / bw (x10⁻³)</td>
<td>5.3 ± 0.3</td>
<td>6.9 ± 0.3*</td>
</tr>
<tr>
<td>HNR</td>
<td>0.67 ± 0.12</td>
<td>-</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>1.8 ± 0.2*</td>
<td>0.8 ± 0.2*</td>
</tr>
<tr>
<td>Inflammation</td>
<td>1.8 ± 0.2*</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>Glomerular changes</td>
<td>1.2 ± 0.3*</td>
<td>0.6 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM. n=number of animals, bw=body weight, kw=kidney weight, HNR=hydronephrotic ratio

* P<0.05 compared with Controls (same genotype)

a P<0.05 compared with wild-type (Controls)

b P<0.05 compared with wild-type (Hydronephrosis)

c P<0.05 compared with SOD1-tg (Controls)

d P<0.05 compared with SOD1-tg (Hydronephrosis)
Table 3. **TGF characteristics in control and hydronephrotic rats during control conditions (Baseline) and after the administration of Tempol.**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Tempol</th>
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<tbody>
<tr>
<td></td>
<td>PT, mmHg</td>
<td>11.9±0.4</td>
</tr>
<tr>
<td></td>
<td>PSF, mmHg</td>
<td>40.2±0.5</td>
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<tr>
<td><strong>Controls</strong></td>
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<td></td>
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<tr>
<td>∆PSF, mmHg</td>
<td>9.2±0.7</td>
<td>7.0±0.5</td>
</tr>
<tr>
<td>TP, nl/min</td>
<td>18.7±1.1</td>
<td>18.3±1.1</td>
</tr>
<tr>
<td>m/n</td>
<td>6/7</td>
<td>4/9</td>
</tr>
<tr>
<td></td>
<td>PT, mmHg</td>
<td>13.9±0.7</td>
</tr>
<tr>
<td></td>
<td>PSF, mmHg</td>
<td>40.5±1.2</td>
</tr>
<tr>
<td><strong>Hydronephrosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆PSF, mmHg</td>
<td>15.2±1.2</td>
<td>9.1±0.6</td>
</tr>
<tr>
<td>TP, nl/min</td>
<td>14.3±0.8</td>
<td>19.7±1.4</td>
</tr>
<tr>
<td>m/n</td>
<td>4/6</td>
<td>4/7</td>
</tr>
</tbody>
</table>

Values are means ± SEM. PT=proximal tubular pressure, PSF=stop-flow pressure, ∆PSF= maximal stop-flow pressure response, TP=turning point, m=animals, n=nephrons.

* P<0.05 compared with control values of same group,

# P<0.05 compared with values of control animals under similar conditions.