Early superoxide scavenging accelerates renal microvascular rarefaction and damage in the stenotic kidney

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Kelsen S, He X, Chade AR. Early superoxide scavenging accelerates renal microvascular rarefaction and damage in the stenotic kidney. Am J Physiol Renal Physiol 303: F576–F583, 2012. First published May 23, 2012; doi:10.1152/ajprenal.00154.2012.—Renal artery stenosis (RAS), the main cause of chronic renovascular disease (RVD), is associated with significant oxidative stress. Chronic RVD induces renal injury partly by promoting renal microvascular (MV) damage and blunting MV repair in the stenotic kidney. We tested the hypothesis that superoxide anion plays a pivotal role in MV dysfunction, reduction of MV density, and progression of renal injury in the stenotic kidney. RAS was induced in 14 domestic pigs and observed for 6 wk. Seven RAS pigs were chronically treated with the superoxide dismutase mimetic tempol (RAS+T) to reduce oxidative stress. Single-kidney hemodynamics and function were quantified in vivo using multidetector computer tomography (CT) and renal MV density was quantified ex vivo using micro-CT. Expression of angiogenic, inflammatory, and apoptotic factors was measured in renal tissue, and renal apoptosis and fibrosis were quantified in tissue sections. The degree of RAS and blood pressure were similarly increased in RAS and RAS+T. Renal blood flow (RBF) and glomerular filtration rate (GFR) were reduced in the stenotic kidney (280.1 ± 36.8 and 34.2 ± 3.1 ml/min, P < 0.05 vs. control). RAS+T kidneys showed preserved GFR (58.5 ± 6.3 ml/min, P = not significant vs. control) but a similar decreases in RBF (293.6 ± 85.2 ml/min) and further decreases in MV density compared with RAS. These changes were accompanied by blunted angiogenic signaling and increased apoptosis and fibrosis in the stenotic kidney of RAS+T compared with RAS. The current study shows that tempol administration provided limited protection to the stenotic kidney. Despite preserved GFR, renal perfusion was not improved by tempol, and MV density was further reduced compared with untreated RAS, associated with increased renal apoptosis and fibrosis. These results suggest that a tight balance of the renal redox status is necessary for a normal MV repair response to injury, at least at the early stage of RVD, and raise caution regarding antioxidant strategies in RAS.

renal artery stenosis; tempol; renal hemodynamics; imaging

RENA L ARTERY STENOSIS (RAS) is a prominent cardiovascular risk factor and the main cause of chronic renovascular disease (RVD), which may progress to chronic kidney disease and lead to end-stage renal disease (16, 40). An important mechanism by which chronic RVD induces renal injury is by promoting renal microvascular (MV) endothelial dysfunction, which may eventually progress to MV remodeling, damage, and loss. A defective microcirculation is an important common pathological feature in chronic renal disease (20). Changes in the glomerular and peritubular microvasculature (26) have been shown to impair renal blood flow (RBF) and promote ischemia and scarring in progressive renal disease (19) irrespective of the primary etiology, supporting a major role for MV disease on the development and progression of renal injury (20).

Reactive oxygen species (ROS) are potent direct vasoconstrictors and enhance the sensitivity to other vasoconstrictors like angiotensin II and endothelin-1. These effects have been demonstrated in virtually every organ, including the kidney, in which ROS are prominent controllers of filtration, vascular, and tubular function (34, 35). A sustained decrease in blood flow to the kidneys, as occurs in chronic RVD, could serve as a potent stimulus for the generation of ROS and oxidative stress. ROS may also act as second messengers and activate numerous proinflammatory and progrowth injurious cytokines. This is likely part of the initial response to injury in RVD but as the disease progresses, it may promote inflammation and fibrosis and contribute to further deterioration of renal function (8).

Using a well-established swine model of RAS (as a surrogate of chronic RVD), we previously showed that superoxide anion, the most abundant renal ROS, is significantly increased in the stenotic kidney and associated with decreased RBF, glomerular filtration rate (GFR), cortical MV rarefaction, and renal fibrosis (8, 10, 48). These results served as basis for our current study, which was designed to test the hypothesis that superoxide anion is a key factor in progression of renal injury by promoting MV dysfunction, damage, and loss in the stenotic kidney. To test this hypothesis, pigs with RAS were chronically administered tempol, a potent superoxide dismutase (SOD) mimic with specific scavenging properties (31). However, dismutation of superoxide could have an unintended deleterious effect by increasing the production and bioavailability of hydrogen peroxide, which may also modulate MV function and induce tissue damage. Therefore, the second goal of the study was to determine whether early scavenging of superoxide might interfere with the responses in the stenotic kidney to an ischemic insult.

METHODS

The Institutional Animal Care and Use Committee at the University of Mississippi Medical Center approved all the procedures. Twenty-one domestic pigs (50–55 kg) were studied after 6 wk of observation. In 14 pigs, unilateral RAS was induced at baseline by placing a local coil inside the main renal artery, which induced gradual development of RAS, as previously described (8, 25). The pigs were then randomized into two groups: those that were not further treated (RAS, n = 7) or those treated with the specific SOD mimetic tempol (2 mmol·l⁻¹·day⁻¹, RAS+T, n = 7) beginning at insertion of the coil and induction of RAS. Tempol (Sigma, St. Louis, MO) is a specific and potent short-acting superoxide radical scavenger, which does not bind nitric oxide (NO) or produce superoxide anion (31). The mixture

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was prepared daily and administered in the drinking water at a dose of 2 mg/kg, based on previous protocols demonstrating that this regimen has a potent antioxidant effect without decreasing blood pressure in normal animals (44). Throughout these 6 wk, blood pressure was continuously measured using telemetry (PhysioTel, Data Sciences) implanted at baseline in the left femoral artery. Mean arterial pressure (MAP) was recorded at 5-min intervals and averaged for each 24-h period. Seven additional pigs were used as controls (normal, n = 7).

At 6 wk, all RAS pigs underwent renal angiography to quantify the degree of RAS. For this, the pigs were anesthetized with intramuscular telazol (5 mg/kg) and xylazine (2 mg/kg), intubated, and mechanically ventilated with room air. Anesthesia was maintained with a mixture of ketamine (0.2 mg·kg⁻¹·min⁻¹) and xylazine (0.03 mg·kg⁻¹·min⁻¹) in normal saline, administered via an ear vein cannula (0.05 ml·kg⁻¹·min⁻¹) and angiography was performed under sterile conditions and fluoroscopic guidance and degree of stenosis were determined as previously described (18). Immediately after angiography, the 9F catheter used for angiography was positioned in the superior vena cava and in vivo helical multidetector computer tomography (MDCT) flow studies were performed for assessment of single-kidney RBF (ml/min), perfusion (ml·min⁻¹·g⁻¹·tissue), and GFR (ml/min), as previously detailed and validated (4, 18). Studies were repeated during suprarenal intra-aortic infusion of endothelium-dependent vasodilator acetylcholine (Ach; 5 μg·kg⁻¹·min⁻¹). Renal vascular resistance was calculated by dividing the MAP (measured at the time of the in vivo studies) and MDCT-derived RBF. Blood was collected from the stenotic and contralateral renal veins and inferior vena cava, and plasma renin activity (PRA, RIA), serum creatinine (spectrophotometry), and hydrogen peroxide (colorimetric assay) were measured.

Following completion of all studies, the pigs were allowed to recover for 2 days (to allow for contrast media washout) and were then euthanized with a lethal intravenous injection of pentobarbital sodium (100 mg/kg; Sleepaway, Fort Dodge Laboratories, Fort Dodge, IA). Kidneys were removed using a retroperitoneal incision and immersed in heparinized saline (10 U/ml). A lobe of kidney was used for micro-CT reconstruction, while another lobe of tissue was removed from one end of the kidney, snap-frozen in liquid nitrogen, and stored at −70°C for subsequent analysis, as previously described (6, 11). Briefly, one to three intracortical arterioles and their branches were tomographically “dissected” in each pig, the three-dimensional path distance (total length) and linear distance (shortest distance between endpoints) of the main branches were calculated, and the tortuosity index was calculated by dividing path distance by linear distance and expressed as a ratio.

Lucigenin chemiluminescence assay. Superoxide production in the renal cortex was measured by Lucigenin luminescence. Tissue (100 mg) was homogenized in RIPA Lysis Buffer System (Santa Cruz Biotechnology, Santa Cruz, CA). Samples were centrifuged at 18,000 g for 20 min and the supernatant was collected for the measurements. Basal tissue oxidative stress was determined by incubating 100 μl of the supernatant with 350 μl PBS and 50 μl Lucigenin (Sigma) at a final concentration of 5 μM. The samples were allowed to equilibrate for 3 min in the dark, and luminescence was measured every second for 5–15 min with a luminometer (Berthold, Oak Ridge, TN). Luminescence was recorded as relative light units per minute (RLU/min). To determine NADPH-stimulated oxidative stress, 100 μl of the supernatant were incubated with 300 μl PBS, 50 μl 1 mM NADPH (Sigma), and 50 μl Lucigenin at a final concentration of 5 μM. An assay of a blank with 450 μl PBS and 50 μl Lucigenin was subtracted from the reading before transformation of the data. The protein concentration was measured using a Pierce (Rockford, IL) protein assay with BSA standards. The data are expressed as RLU per minute per milligram protein.

Hydrogen peroxide assay. Plasma (from renal vein from the stenotic kidney) levels of hydrogen peroxide were measured using a colorimetric assay (BioVision, Mountain View, CA). Samples were centrifuged at 1,000 g for 15 min and filtered through a 10-Ka mw spin filter (BioVision) to remove all proteins. The data are expressed as RLU per minute per milligram protein.

Histology. Midhilar 5-μm cross sections of each kidney (1 per tissue) levels of hydrogen peroxide were measured using a colorimetric assay (BioVision, Mountain View, CA). Samples were centrifuged at 1,000 g for 15 min and filtered through a 10-Ka mw spin filter (BioVision) to remove all proteins. Then, 25 μl of each filtrate were added in duplicate into each well of a 96-well plate and 25 μl of assay buffer were added to them. Standards were generated by adding 0.1 mM freshly prepared H₂O₂ at concentrations of 0, 1, 2, 3, 4, 5 nmol/well. Fifty microliters of a reaction mixture containing OxiRed Probe and horseradish peroxidase were then added to all wells and incubated for 10 min at room temperature. Optical density was measured at 570 nm and background was corrected by subtracting the value derived from the 0-nmol standard. The results are expressed in picomoles per milliliter.

Growth factors. Renal expression of VEGF, HIF-1α, and TNF-α was quantified in cortical homogenates of the stenotic kidney by Western blotting, following standard procedures (18, 21).

Apoptosis. The fraction of apoptotic cells was calculated in 15 tubule-interstitial and glomerular randomly selected regions in the stenotic (or normal) kidney of each pig. Apoptosis was expressed as a percent calculated by the number of apoptotic cells out of the total number of cells per field. Furthermore, the renal expression of apoptotic mediators such as Bax, caspase-3 and -8, and AIF was quantified in the stenotic kidney by Western blotting (18, 21).

Histology. Midhilar 5-μm cross sections of each kidney (1 per animal) were examined using a computer-aided image-analysis program (ImageJ, NIH, and Adobe Photoshop, San Francisco, CA). In each representative slide, trichrome staining was semi-automatically quantified in 15–20 fields by the computer program, expressed as percentage of staining of total surface area, and the results from all fields were averaged (18). Glomerular score (percentage of sclerotic glomeruli) was assessed by recording the number of sclerotic glomeruli out of 100 counted glomeruli, as previously described (18).

Statistical analysis. Results are expressed as means ± SE. Comparisons within groups were performed using paired Student’s t-test, and among groups using one-way ANOVA, with Bonferroni correction for multiple comparisons. Statistical significance was accepted for P ≤ 0.05.
Table 1. Mean arterial pressure, degree of stenosis, serum creatinine, and basal single-kidney hemodynamics and function in normal, RAS, and RAS pigs + T

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal n = 7</th>
<th>RAS n = 7</th>
<th>RAS+T n = 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>99.0 ± 3.0</td>
<td>132.1 ± 2.9*</td>
<td>127.2 ± 4.2*</td>
</tr>
<tr>
<td>Degree of stenosis, %</td>
<td>0.0 ± 0.0</td>
<td>74.6 ± 6.8*</td>
<td>71.9 ± 7.4*</td>
</tr>
<tr>
<td>Plasma renin activity, ng·mL⁻¹·h⁻¹</td>
<td>0.20 ± 0.04</td>
<td>0.20 ± 0.03</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>Serum creatinine, μmol/l</td>
<td>114.0 ± 5.3</td>
<td>144.1 ± 8.8*</td>
<td>132.6 ± 7.1*</td>
</tr>
<tr>
<td>Renal vascular resistance, mmHg·ml⁻¹·min⁻¹·g⁻¹</td>
<td>0.19 ± 0.02</td>
<td>0.62 ± 0.1*</td>
<td>0.6 ± 0.1*</td>
</tr>
<tr>
<td>Renal volume, ml</td>
<td>123.6 ± 8.2</td>
<td>67.2 ± 5.5*</td>
<td>71.7 ± 4.0*</td>
</tr>
<tr>
<td>Medulla</td>
<td>37.4 ± 2.4</td>
<td>23.5 ± 2.4*</td>
<td>22.5 ± 2.3*</td>
</tr>
<tr>
<td>Renal blood flow, ml/min</td>
<td>527.8 ± 54.5</td>
<td>280.1 ± 36.8*</td>
<td>293.6 ± 85.2*</td>
</tr>
<tr>
<td>Cortex</td>
<td>3.5 ± 0.4</td>
<td>3.3 ± 0.4</td>
<td>3.5 ± 1.2</td>
</tr>
<tr>
<td>Medulla</td>
<td>2.7 ± 0.4</td>
<td>2.7 ± 0.5</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml/min</td>
<td>55.9 ± 5.8</td>
<td>34.2 ± 3.1*</td>
<td>58.5 ± 6.3*</td>
</tr>
</tbody>
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Values are means ± SE. Mean arterial pressure (average of last 2 wk before computer tomography studies), degree of stenosis, serum creatinine, and basal single-kidney hemodynamics and function (means ± SE) in normal, renal artery stenosis (RAS), and RAS pigs treated with tempol (RAS+T). *P < 0.05 vs. normal. †P < 0.05 vs. RAS.

RESULTS

General characteristics. Serum creatinine, MAP, and the angiographic degree of stenosis were similar in RAS and RAS+T animals and significantly greater compared with normal untreated controls, while PRA was similar among the groups (Table 1). RAS showed a significant increase in renal superoxide and hydrogen peroxide (Fig. 1). However, tempol treatment normalized superoxide in the stenotic kidney and further increased hydrogen peroxide bioavailability, suggesting effective superoxide scavenging and degradation.

MDCT-derived renal hemodynamics. Renal volume, RBF, and regional perfusion were similarly decreased as renal vascular resistance increased in RAS and RAS+T compared with normal untreated controls (P < 0.05 vs. RAS and RAS+T, Table 1). Tempol treatment prevented the decrease in GFR in RAS+T (Table 1). However, RBF, GFR, and perfusion responses to infusion of Ach were blunted response in both RAS and RAS+T kidneys, suggesting significant and persistent MV endothelial dysfunction. These were accompanied by a similar attenuation in the renal expression of p-eNOS in both RAS and RAS+T kidneys (Fig. 2).

MV three-dimensional architecture and growth factors. Micro-CT reconstruction of the renal MV architecture showed that the density and distribution of microvessels in the stenotic kidney in RAS were significantly decreased across the renal cortex (inner, middle, and outer cortex), while they were preserved in the medulla. Notably, the MV density and vascular volume fraction were further decreased in RAS+T kidneys (Fig. 3), affecting not only the cortex but also the medulla, suggesting a deleterious effect of chronic superoxide scavenging on the microvasculature of the stenotic kidney. MV tortuosity was similarly increased in RAS and RAS+T kidneys (1.72 ± 0.08 and 1.65 ± 0.1) compared with normal controls (0.99 ± 0.12, P < 0.05 vs. RAS and RAS+T). The decreased MV density in RAS+T kidneys was accompanied by a similar decrease in VEGF (to that observed) in untreated RAS compared with normal kidneys, but a further decrease in the expression of HIF-1α, suggesting blunted angiogenic signaling after tempol administration (Fig. 3).

Apoptosis. The fraction of apoptotic cells was increased at both tubule-interstitial and glomerular compartments in the RAS kidney, accompanied by augmented expression of proapoptotic TNF-α, caspase-3 and -8, and Bax. RAS+T showed a further increased expression of proapoptotic factors and fraction of renal apoptotic cells (Fig. 4, A and B).

Renal morphology. The RAS and RAS+T kidneys showed a significant glomerulosclerosis, accompanied by increased perivascular and tubulointerstitial fibrosis compared with normal controls (Fig. 5). Notably, tempol treatment resulted in a strong trend toward an increase in renal fibrosis compared with RAS as well (P = 0.076 vs. RAS; Fig. 5).
DISCUSSION

The current study shows that scavenging of superoxide anion in experimental RVD by tempol administration preserved basal GFR but did not have beneficial effects on RBF, regional perfusion, or endothelial function in the stenotic kidney. Furthermore, MV density (both in cortex and medulla) was further decreased and accompanied by blunted expression of angiogenic factors and augmented expression of inflammatory and apoptotic mediators, increased apoptotic cells throughout the renal compartments, and a trend toward increased renal fibrosis after treatment with tempol. These dual effects of superoxide scavenging suggest that a tight balance of the renal redox status is necessary for a normal physiological response to injury in the stenotic kidney, at least at the early stage of RVD.

ROS are well-known for their role in the pathogenesis and progression of several diseases such as hypertension (33), diabetes (15), and atherosclerosis (7). Vast experimental evidence supports the notion that ROS play a role in promoting functional and structural injury in the long term and that antioxidant strategy is beneficial in several models of cardiovascular and renal disease (10, 12, 44, 49). On the other hand, evidence derived from clinical studies is not conclusive, since a few studies have shown benefits from antioxidants but several trials have shown mixed results (13, 47). The reasons for disparate results in experimental and clinical studies are not

Fig. 2. Responses to intrarenal infusion of acetylcholine (Ach; expressed in %change from baseline, left) and renal protein expression and quantification of phosphorylated endothelial nitric oxide synthase (eNOS; right) in normal, RAS, and RAS pigs treated with tempol (RAS+tempol). Responses to Ach and eNOS expression were similarly decreased in RAS and RAS+tempol suggesting persistence of endothelial dysfunction in the stenotic kidney after tempol administration. *P < 0.05 vs. normal.

Fig. 3. Representative 3-dimensional micro-computer tomography reconstruction (top), quantification of the microvascular (MV) density and vascular volume fraction (bottom left and middle), and renal expression of hypoxia-induced factor (HIF)-1α and vascular endothelial growth factor (VEGF) in normal, RAS, and RAS kidneys treated with tempol (RAS+tempol). Tempol administration further deteriorated angiogenic signaling and further decreased MV density in the stenotic kidney compared with untreated RAS. *P < 0.05 vs. normal. †P < 0.05 vs. RAS.
clear, but might be related to the type of antioxidant strategy, timing, and length of its use, or the type, severity, and stage of the disease. We recently showed that chronic supplementation of antioxidant vitamins C and E protected renal function and MV density in the stenotic kidney (10, 48). Although vitamins C and E have a lower affinity to ROS, they have been shown to prevent oxidation of LDL cholesterol, reduce superoxide anion production, and promote its scavenging, and inhibit ROS-sensitive signaling pathways involved in cytokine release and cell proliferation (27, 30), supporting additional effects beyond their antioxidant power that promote renoprotection. Furthermore, we reported that acute antioxidant intervention using tempol provides only limited functional beneficial effects in the stenotic kidney compared with chronic vitamins (5), underscoring ideas that decreasing or preventing oxidative stress-induced structural damage plays an important part in the pathogenesis of renal injury in RVD. However, whether chronic and specific scavenging of superoxide anion is sufficient to protect renal MV architecture and function in the stenotic kidney is unknown.

In the current study, we chronically treated RAS pigs from the onset of the disease with tempol, a potent and specific SOD mimetic scavenger (31) to aggressively remove renal superoxide and prevent potentially deleterious effects on the renal microcirculation. Superoxide anion is the most abundant ROS in the kidney and has high affinity for NO that is three times faster than the reaction of superoxide with SODs, and 10,000 times faster than its reaction with antioxidant enzymes (17). The dose we used in our study was effective in reducing renal superoxide to normal levels without modifying blood pressure, avoiding a potential major confounding effect on renal function. Tempol does not act as a catalase mimetic and does not bind to NO or produce superoxide anion (31). The scavenging resulted in a preservation of GFR possibly due to a greater effect of tempol in the afferent than the efferent arteriole (36, 43) (likely via increased bioavailability of NO), leading to a relative increase in efferent arteriolar resistance and augmented ultrafiltration coefficient. The similar expression of eNOS in the stenotic kidney of RAS and RAS+T pigs suggests that generation of NO was unchanged but that bioavailability could have been improved by enhanced superoxide scavenging in RAS+T, which may have been at least sufficient to preserve basal GFR. Furthermore, additional mechanisms that may have contributed to the preserved GFR in RAS+T could be a tempol-mediated decreased renal sympathetic nerve activity (37) and/or attenuation of tubule-glomerular feedback (34). Despite preserved GFR, there was a noticeable lack of improvement on RBF, regional perfusion, or serum creatinine, which were similar to untreated RAS pigs. We previously showed similar distinct effects on GFR after an acute intrarenal administration of tempol in this model (5). The lack of an effect on RBF suggests complicated interactions of the pre- and postglomerular vasculature that may have shifted the resistance tissue target. However, the reasons behind the preserved GFR with low RBF in RAS+T are not entirely clear.

The unchanged RBF and perfusion were associated with further reductions in cortical and medullary MV density and increased renal damage in the RAS+T stenotic kidney. Finding a further decrease in MV density associated with preserved GFR but a similar decrease in RBF suggests that tempol administration from the onset of RAS favored the recruitment of preexistent vessels and induced a sufficient vasodilatation to sustain renal perfusion at baseline to compensate a chronic obstruction of blood flow with a greater reduction of MV density. The decrease in MV density in RAS kidneys and the further decrease in RAS+T animals are likely structural rather
than functional, because our sample preparation methods allow for maximal MV relaxation before fixation. Despite evidence suggestive of some vascular sprouting [e.g., increased MV tortuosity (6, 11)], the blunted expression of angiogenic mediators (further decreased by tempol) may have severely limited the potential for MV proliferation, development, and repair in the RAS+T kidney. Indeed, tempol administration was accompanied by a further decrease in the renal expression of proangiogenic HIF-1α and a similar decrease in VEGF compared with untreated RAS. We previously showed that these proangiogenic mediators progressively decreased in the stenotic kidney and that the decrease parallels MV rarefaction and deterioration of renal function (18, 48). A further deterioration of renal angiogenic signaling and amplified progression of MV rarefaction suggests that early removal of superoxide in RAS+T was partly harmful for the stenotic kidney.

We recently showed that endogenous antioxidant defenses such as SOD, glutathione peroxidase, and catalase are blunted in the stenotic kidney (8), indicating a marked imbalance between production and degradation of ROS. Tempol administration effectively scavenged and degraded superoxide but led to a significant increase in hydrogen peroxide bioavailability in RAS+T, in a context of decreased activity of renal catalase in the stenotic kidney (8). Hydrogen peroxide is a potent vasodilator (46) and its abundance may have played a role in the sustained renal perfusion in the RAS+T kidney. However, it is also a potent stimulus for activation of injurious cytokines and a promoter of deleterious mechanisms such as inflammation, apoptosis, and fibrosis. Indeed, hydrogen peroxide has been shown to be a powerful profibrotic stimulus (23, 39), which may explain the strong trend toward an increase in tubulointerstitial fibrosis (with a similar degree of glomerulosclerosis) in RAS+T compared with untreated RAS kidneys. Thus, the increased availability of hydrogen peroxide in the stenotic kidney may partly explain the augmented renal damage after tempol administration. The additional role of hydrogen peroxide is underscored by a similar PRA among the groups that may partly exclude an augmented activation of the renin-angiotensin system as a culprit of the increased renal damage in RAS+T. However, we cannot rule out the possibility that renal vasoconstriction and damage in the stenotic kidney may have been partly mediated by the local renal renin-angiotensin system (6, 45) in our study.

Apoptosis is one of the main mechanisms by which endothelial cells are killed when facing ischemia (32) and a key promoter of MV rarefaction (42). We recently showed that apoptosis is increased in the stenotic kidney (9, 21). We observed that the fraction of apoptotic cells was further increased in RAS+T kidneys, evident throughout the renal vascular, tubular, and glomerular compartments. Increased apoptosis was accompanied by a similar increased expression of TNF-α in both RAS and RAS+T kidneys. TNF-α is a potent proinflammatory cytokine with proapoptotic effects both directly and mainly via stimulation of caspases (2, 28, 29). In addition, the expressions of caspase-3 and -8 were significantly increased in RAS+T compared with untreated RAS and
normal controls, supporting the notion of prosapoptotic effects by tempol administration, likely via increasing hydrogen peroxide (22). The resulting disturbance of superoxide/NO balance in the RAS+T kidney may have also contributed to enhance apoptosis via mitochondrial dysfunction (1). Furthermore, AIF, the main controller of caspase-independent apoptosis (38), was significantly increased in RAS+T kidneys. AIF has been shown to play a central role in hydrogen peroxide-driven cell death (38), indicating that both caspase-dependent and -independent pathways were activated in the RAS+T kidney and may have contributed to loss of vascular cells.

Future studies using other antioxidants such as apocynin or xanthine-oxidase inhibitors will further establish whether antioxidant interventions or specifically tempol from the onset of RAS are the reason behind the mixed effects observed in the current study. Superoxide plays an important role in many enzymatic processes such as the phosphorylation and activation of numerous protein kinases acting as a central signaling molecule (3). The mixed effects after tempol administration suggest that early scavenging and dismutation of superoxide (and/or the subsequent increased hydrogen peroxide) may accelerate the progression of renal injury in the stenotic kidney. Because tempol led to a significant accumulation of hydrogen peroxide, future studies designed to decrease hydrogen peroxide in the stenotic kidney would further dissec the relative contributions of superoxide vs. hydrogen peroxide accumulation in accelerating renal MV and parenchymal injury, both from the onset and after established renal damage at a later stage of RAS. Furthermore, although it has been shown that tempol does not induce nephrotic effects on normotensive animals (41), it would be of interest to determine in future studies whether chronic superoxide scavenging can induce MV damage in the normal kidney.

In summary, the current study shows that tempol administration from the onset of RAS protected the stenotic kidney in a limited fashion. Despite preserving GFR, RBF and perfusion were not improved and renal MV density was further reduced compared with untreated RAS, associated with accelerated apoptosis and fibrosis. These effects suggest that a tight balance of the renal redox status is necessary to allow a normal physiological response to injury, at least at the early stage of RVD, and may open new questions of the role of superoxide as an important participant in the response to injury of the stenotic kidney. Although most experimental evidence shows an overwhelming support of antioxidants as protective agents in renal disease, the clinical data are still controversial, and the current work may raise caution on the use and timing of application of antioxidant interventions in patients with RVD.

REFERENCES


