Treatment with pyrrolidine dithiocarbamate improves proteinuria, oxidative stress, and glomerular hypertension in overload proteinuria

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INCREASED URINARY EXCRETION of protein is a marker of poor outcome for chronic renal disease (11). The mechanisms involved in the renal damage induced by proteinuria include increased glomerular permeability to proteins which trigger tubulointerstitial (TI) oxidative stress, inflammation, and scarring (30); over time, the magnitude of proteinuria correlates with that of TI injury and the rate of declining renal function (6, 7, 30).

Unbalanced generation of reactive oxygen species (ROS) plays a role in the progression of different renal diseases by the local nephrotoxic potential of oxidative stress and nitrosating reactions (49). Proteinuria is a well-known insult associated with increased oxidative stress and inflammation in renal tissue (1, 14, 15). In this regard, exposing cultured proximal tubular cells to proteins increased the production of ROS with the subsequent activation of NF-κB and production of proinflammatory molecules (52, 53). Additionally, increased protein excretion is associated with the production of vasoactive mediators including angiotensin II and endothelin-1 (19, 42); these vasoconstrictors also induce oxidative stress, NF-κB activation, and inflammation (19). Therefore, blockade of oxidative stress and the subsequent activation of NF-κB may be effective approaches to disrupt the process of TI injury. Supporting this hypothesis, treatment of rats with the antioxidant and NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) has been reported to be beneficial in adriamycin-induced nephropathy (31), subtotal renal ablation (17), rats overexpressing the human renin and angiotensinogen genes (25), and spontaneously hypertensive rats (33). Inhibition of NF-κB activation by gene transfer of truncated IκB has also been reported to protect rats with overload proteinuria (41).

A key unanswered question is how blockade of NF-κB activation alters glomerular hemodynamics in these models. It is known, for example, that after the loss of kidney functional units, surviving nephrons activate hemodynamic changes to preserve renal function; however, in the long term, these physiologic alterations activate pathogenic pathways that lead to renal structural damage in a self-perpetuating circle (8). Recently, our group reported that TI inflammation and microvascular injury induce profound effects on glomerular hemodynamics, resulting in impaired renal autoregulation and the development of glomerular hypertension (16, 36, 37, 43). The development of these lesions increases proteinuria and accelerates renal injury; in contrast, measures that block these changes reduce glomerular pressure and protein excretion, suggesting an important mechanism for renoprotection (35).

Yoshioka and Ichikawa (51) showed that glomerular hypertension and cortical vasoconstriction occur soon after the induction of oxidative stress induced by the infiltration of...
METHODS

Reagents and drugs were obtained from Sigma (St. Louis, MO) until established otherwise. All animal procedures were performed in accordance to the Mexican Federal Regulation for animal experimentation and care (NOM-062-ZOO-2001) and were approved by Bioethics and Investigation Committees of Instituto Nacional de Cardiología Ignacio Chavez. Male Sprague-Dawley rats (300–350 g body wt) were used in all experiments. We included the following experimental groups: (1) saline: rats received 6 ml daily intraperitoneal injections of saline solution (SS; 0.09%; n = 7), 2) AO: induced by daily intraperitoneal injections of BSA (2 g/6 ml SS, n = 10), and 3) AO+PDTC: PDTC, a nonspecific NF-κB inhibitor with antioxidant properties (200 mg/kg·day−1 sc; n = 10), was administered concomitant to AO.

AO and pharmacological treatment were given for 2 wk. Proteinuria was measured at baseline and after 3, 5, and 13 days of follow-up. Systolic blood pressure (SBP) was measured at baseline and after 5 and 13 days of follow-up. Micropuncture studies were performed at the end of the study.

SBP was measured by tail-cuff sphygmomanometer (Narco Biosystems, Houston, TX) in animals preconditioned during 1 wk before blood pressure measurements. Twenty-four-hour proteinuria was determined by turbidimetry (20).

Micropuncture Studies

Animals were anesthetized with pentobarbital sodium (30 mg/kg ip) and placed on a thermoregulated table at 37°C. Trachea, jugular veins, femoral arteries, and the left ureter were catheterized with polyethylene tubing (PE-240, PE-50, and PE-10), and the left kidney was exposed. Mean arterial pressure (MAP) was monitored with a pressure transducer (model p23 db; Gould, San Juan, PR) and recorded on a polygraph (Grass Instruments, Quincy, MA). Blood samples taken during the experiment were replaced with blood from a donor rat. Rats were maintained under euclidean conditions by infusion of 10 ml/kg body wt of isotonic rat plasma during surgery, followed by an infusion of 10% polyfructosan, at 2.2 ml/h (Inutest, Fresenius Pharma Austria GmbH Linz, Austria). After 60 min, five to six 3-min collection samples of proximal tubular fluid were obtained to determine flow rate and polyfructosan concentration. Infratubular pressure under free-flow and stop-flow conditions and peritubular capillary pressure were measured in other proximal tubules with a servo-null device (Servo Nulling Pressure System, Instrumentation for Physiology and Medicine, San Diego, CA). Care was taken during the procedure to avoid micropuncture of any nephrons that had visible proteinaceous casts in their tubules. Polyfructosan was measured in plasma samples and in the fluid collected from individual nephrons according to methods given elsewhere (12, 47). Glomerular colloid osmotic pressure was estimated from protein concentration from blood of the femoral artery (Ca) and surface effenter arterioles (Ce) determined according to the method of Viets et al. (46). MAP, glomerular filtration rate (GFR), glomerular capillary hydrostatic pressure (PGC), single-nephron plasma flow, afferent (AR) and efferent (ER) resistances, and ultrafiltration coefficient (Kf) were calculated according to equations given elsewhere (5).

Histological Studies

After the micropuncture study, right kidney was excised, and the renal cortex was separated and frozen at −80°C until processing. Left kidneys were washed by perfusion with PBS, fixed with 4% paraformaldehyde, excised, and weighed. Renal biopsies were embedded in paraffin.

Oxidative Stress Markers

Immunohistochemistry of p65, 4-hydroxynonenal, and 3-nitrotyrosine. Tissues were processed as previously reported (9, 50). In brief, kidney sections (3 μm) were deparaffinized and then boiled in Declere to unmask antigen sites; the endogenous activity of peroxidase was quenched with 0.03% H2O2 in absolute methanol. Kidney sections were incubated overnight at 4°C with 1:100 dilution of anti-p65 (Santa Cruz Biotechnology, Santa Cruz, CA), 1:200 dilution of anti-4-hydroxynonenal (4-HNE), and 1:70 dilution of anti-3-nitrotyrosine (3-NT) antibodies in PBS. Following removal of the primary antibodies and repetitive rinsing with PBS, slides were incubated with a 1:500 dilution of biotinylated goat anti-IgG secondary antibody. Bound antibodies were detected with avidin-biotinylated peroxidase complex ABC-kit Vectastain and diaminobenzidine substrate. After appropriate washing in PBS, slides were counterstained with hematoxylin. All sections were incubated under the same conditions with the same concentration of antibodies and in the same running, so the immunostaining was comparable among the different experimental groups. For the negative control, preimmune goat serum was used instead of the primary antibodies (9, 50). All specimens were examined by light microscopy Axiosvert 200M (Carl Zeiss, Jena, Germany) analyzing cortex and medulla independently. For automated morphometry analysis, the percentage of immunopositive brown area (immunopositive area/total image area × 100) was determined with a computerized image analyzer KS-300 3.0 (Carl Zeiss) by averaging five random fields per kidney at ×100 magnification. Results were expressed as percentage of area.

Inflammatory Markers

TI monocytes/macrophages infiltration was evaluated with the immunoperoxidase technique using an anti-rat monocyte/macrophage (CD68) monoclonal antibody (Chemicon International, Temecula, CA). Negative control consisted of slides incubated with nonrelevant antisera. TI inflammation was evaluated in 30 nonoverlapping ×400 (235.42 × 316.02 μm) cortical fields, excluding glomeruli, per biopsy. Results were expressed as positive cells per 0.074 mm² using the mean of the 30 fields.

NOS II-positive expression was evaluated by indirect streptavidin-biotin-peroxidase using a specific monoclonal antibody (BD Transduction Laboratories) and counterstained with hematoxylin in (4). Ten nonoverlapping fields of cortex (×10) per biopsy were analyzed using Image-Pro-Plus 5.0 (Media Cybernetics, Silver Spring, MD) and Adobe Photoshop 7 (Adobe Systems, San Jose, CA). Positive brown color areas were selected and quantified in pixel units and the number of positive areas was expressed as a fraction of the total area (positive brown areas divided by overall field area). The mean fractional amount of positive brown areas was obtained by averaging the values from 10 examined fields. Results were expressed as positive cells per 0.074 mm².

Arteriolar morphology. Four-micrometer renal sections were assessed by indirect peroxidase immunostaining for α-smooth muscle actin (DAKO, Carpinteria, CA). Sections incubated with normal
rabbit serum were used as negative controls. Afferent arteriole morphology was quantified as previously described (34, 36, 37). In brief, arterioles were identified by their location adjacent to the vascular pole of the glomerular tuft. For each arteriole, the outline of the vessel and its internal lumen (excluding the endothelium) was generated using computer analysis (Image-Pro Plus 5.0, Media Cybernetics) to calculate the total medial area (outline — inline), in 10 arterioles per biopsy. The media/lumen ratio was calculated by the outline/inline relationship. Quantifications were performed blinded at $\times 1,000$.

### Proteinaceous Casts Quantification

Casts were examined in 3-μm sections stained with Masson’s trichrome stain in 30 cortical and 30 medullar nonoverlapping fields at $\times 200$ magnification using Image-Pro-Plus 5.0 (Media Cybernetics). The extent of tissue containing blocked tubules filled with protein was quantified as pixels per field by determining the proportion of stained structures in each compartment (cortex or medulla). The mean value in an individual section was obtained by averaging the 30 fields in each compartment.

### Renal Cortical and Medullary Activity of Antioxidant Enzymes

Renal cortex and medulla obtained from right kidneys were individually homogenized in a Polytom (model PT 2000, Brinkmann, Westbury, NY) for 10 s in cold 50 mM potassium phosphate, 0.1% Triton X-100, pH 7.0. The homogenate was centrifuged at 19,000 g and 4°C for 30 min and the supernatant was separated to measure total protein and the activities of catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), and total superoxide dismutase (SOD).

Total SOD activity in renal cortex and medulla homogenates was assayed spectrophotometrically at 560 nm by a previously reported method using NBT as the indicator reagent (3). The amount of protein that inhibited NBT reduction to 50% of maximum was defined as one unit of SOD activity. Results were expressed as units per milligram of protein. CAT activity in renal cortex was assayed by a method based on the disappearance of 30 mM H$_2$O$_2$ at 240 nm (3) and the data were expressed as k/mg protein. GPX activity in renal cortex and medulla was measured at 340 nm using GR and NADPH in a coupled reaction (3). One unit of GPX was defined as the amount of enzyme that oxidizes 1 μmol NADPH/min. Data were expressed as units per milligram of protein. GR activity in renal cortex and medulla was assayed using oxidized glutathione as substrate and measuring the disappearance of NADPH at 340 nm (3). One unit of GR was defined as the amount of enzyme that oxidizes 1 μmol NADPH/min. Data were expressed as units per milligram of protein.

### Statistical Analyses

Values are expressed as means ± SE. Values from the respective three groups were analyzed by one-way ANOVA. When a $P$ value determined by ANOVA was <0.05, posttest comparisons were made using the Bonferroni test. The relationship between variables was assessed by correlation analysis. GraphPad Prism 5 software (San Diego, CA) was used to perform statistical analysis.

### RESULTS

#### General Parameters

**Proteinuria and SBP.** Administration of saline alone had no effect on proteinuria or SBP, with values similar to baseline throughout the period of the study (Fig. 1).

Rats receiving AO developed heavy proteinuria which peaked at day 3 and tended to decrease in the subsequent days of the experiment, although it remained close to 50-fold higher compared with saline control group at day 13 ($P < 0.01$ vs. saline). In addition, AO resulted in an increase in SBP that reached significance at day 13 ($P < 0.001$ vs. saline).

Treatment with PDTC in AO rats was associated with significantly less proteinuria compared with AO group and was not significantly different from the saline control group. In addition, PDTC treatment prevented the increase in SBP observed at day 13 (Fig. 1).

#### Glomerular Hemodynamics

The results obtained during micropuncture studies performed at the end of the study are depicted in Table 1 and Figs. 1 and 2. MAP was significantly higher in the group administered BSA alone; treatment with PDTC tended to reduce MAP, although the difference did not reach statistical significance. Administration of saline alone did not modify renal hemodynamics parameters (Table 1): whole GFR (Fig. 1), single-nephron GFR (SNGFR), glomerular pressure (Fig. 2), glomerular plasma flow, AR and ER, and $K_t$ had similar values as normal rats as previously reported by our group (43). In contrast, AO was associated with a significant decrement of whole GFR (−29%) and a significant rise of glomerular pressure. PDTC treatment maintained whole GFR similar to the control saline group; in addition, the rise of glomerular pressure induced by AO was partially prevented, being significantly lower compared with AO alone, but still significantly higher compared with the saline controls.

#### Histological Findings

**Kidney/body weight ratio.** AO was associated with an increased renal mass compared with control saline rats (SS: 0.41 ± 0.01; AO: 0.55 ± 0.01; $P < 0.001$). PDTC (0.38 ± 0.02) significantly prevented the enlargement of renal mass induced by AO ($P < 0.05$ vs. AO).

**Immunohistochemistry of oxidative stress markers in renal cortex and medulla.** P65 immunostaining, a marker of NF-kB activation, was increased significantly in both compartments of AO group; PDTC treatment prevented these increments (Figs. 3 and 4).

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**Fig. 1.** Proteinuria (left) and systolic blood pressure (middle) follow-up. Saline ($n = 9$); $\uparrow$, albumin overload (AO; $n = 10$); $\odot$, AO + pyrroline dithiocarbamate (PDTC) treatment ($n = 10$). Right: whole glomerular filtration rate (GFR) at day 14. Data are means ± SE. *$P < 0.05$ vs. saline. **$P < 0.05$ vs. AO.
Oxidative stress was also evident in the AO group; immunostaining for 3-NT- and 4-HNE-modified proteins increased markedly in both renal cortex and medulla relative to saline controls. The increase in both 4-HNE and 3-NT immunostaining was prevented by PDTC (Figs. 3 and 4).

**Immunohistochemistry of inflammatory markers in renal cortex.** As previously reported, AO induced TI inflammation (1, 14); moreover, a marked increment of NOS II expression was also observed (Figs. 3 and 4). A significant thickening of the vascular wall of preglomerular vessels, as reflected by the media-to-lumen ratio, was noted as well. Administration of PDTC to protein overload animals prevented the inflammation and ameliorated NOS II overexpression. Preglomerular vascular structure was also preserved.

**Proteinaceous casts quantification.** Table 2 shows the results of the quantification of proteinaceous casts. There were numerous casts observed in both the cortex and medulla of AO rats. Saline control-treated rats had a negligible presence of casts in the cortex and medulla. PDTC treatment to AO rats significantly prevented the formation of proteinaceous casts in both the cortex and medulla.

**Activity of Antioxidant Enzymes in Renal Cortex and Medulla**

Cortical and medullary antioxidant enzyme activities are shown in Table 3. Consistent with a state of oxidative stress, the renal cortex and medulla of AO rats had decreased CAT, GPX, GR, and total SOD activities. This decrease in antioxidants reached statistical significance for CAT and GPX in the cortex and for CAT, GPX, and SOD in medulla. In the cortex, PDTC treatment prevented the decrease in GPX; moreover, GR activity in the AO+PDTC group was significantly higher from those in AO group. In the medulla, PDTC treatment totally prevented the decrease of GPX and GR and partially maintained SOD activity, while CAT activity remained reduced compared with the saline control group.

**Correlations**

The following correlations were noted for the data obtained from the renal cortex: p65 vs. 3-NT ($r = 0.93$, $P < 0.0001$; Fig. 4), p65 vs. 4-HNE ($r = 0.88$, $P < 0.0001$), p65 vs. CD68+ cells ($r = 0.89$, $P < 0.0001$), p65 vs. NOS II expression ($r = 0.73$, $P = 0.0003$), p65 vs. media-to-lumen (M/L) ratio ($r = 0.57$, $P = 0.0007$), CD68+ cells vs. M/L ratio ($r = 0.67$, $P < 0.0001$), M/L ratio vs. glomerular pressure ($r = 0.77$, $P < 0.0001$), and glomerular pressure vs. proteinuria ($r = 0.64$, $P = 0.0003$).

**DISCUSSION**

In the current investigation, we tested the hypothesis that oxidative stress and inflammation induced by proteinuria might cause microvascular damage and glomerular hemodynamic changes that in turn could accelerate renal injury. We utilized the AO model, which has previously been shown to result in the tubular generation of ROS with the subsequent synthesis and secretion of inflammatory mediators (24, 52). Our primary new finding was that this model results in an afferent arteriolopathy and glomerular hypertension. Most importantly, we demonstrated that blockade of oxidative stress and NF-κB activation reduced this process, leading to a preservation of afferent arteriolar normal structure, prevention of glomerular hypertension, and amelioration of proteinuria. These findings support the notion that afferent arteriole remodeling plays a fundamental role in the genesis and/or perpetuation of glomerular hypertension (35) induced by intense proteinuria.

Previous studies documented a role for oxidative stress and inflammation in renal progression in models of proteinuric renal diseases (1, 14, 17, 31). NF-κB activation plays an important role in this series of events and is highly stimulated by ROS (39). Therefore, maneuvers that reduce oxidative stress and/or prevent the activation of NF-κB are
associated with a better renal outcome (17, 25, 31, 33). In this regard, dithiocarbamates are antioxidants which are potent inhibitors of NF-κB; the most effective NF-κB inhibitor is the pyrrolidine derivative of dithiocarbamate (PDTC) as a result of its ability to traverse the cell membrane and its prolonged stability at physiological pH (45). The potential for modulating both cell activation and the effects of oxidants with PDTC suggests that these agents may offer therapeutic benefit in conditions in which activation of NK-κB plays a major role.

In the present studies, NF-κB p65 subunit staining showed a strong positive correlation with the markers of oxidative stress 3-NT ($r = 0.93$, $P < 0.0001$; Fig. 4) and 4-HNE ($r = 0.88$, $P < 0.0001$). It is well-known that oxidative stress results when increased oxidant synthesis overcomes cellular antioxidant capacity; therefore, we evaluated the renocortical antioxidant capacity in AO rats. Intense proteinuria was associated with a significant decrement in the activity of the antioxidant enzymes SOD, CAT, GR, and GPX; remarkably, treatment with PDTC prevented these changes indicating that antioxidant activity resulted from NF-κB inhibition.

In concert with oxidative stress, AO induced renal inflammation. Inflammatory cell infiltration was documented by the significant increment of CD68 and NOS II immunostaining. Treatment with PDTC decreased the infiltration of inflammatory cells and NOS II overexpression, whose gene is induced by NF-κB. The pathophysiological impact of increased NO derived from NO-κB activation (16, 18, 25, 29, 43) prevented the vascular injury, independent of arterial hypertension. We found that AO induced mild arterial hypertension. Hypertension is not a finding in female Lewis rats with AO (1) but it has been reported by other groups in Sprague-Dawley rats (2).

Massive proteinuria induced a significant decrement of whole GFR ($-29\%$) in the present investigation, which contrasts with two previous studies that found no changes in GFR or renal plasma flow in this model (21, 48). However, in those studies BSA was administered for only 3 to 5 days (21, 48), whereas in our study it was continued for 2 wk. Despite the fall in whole GFR, SNGFR was numerically but not significantly higher compared with the control saline group. Previous studies demonstrated that in AO rats proteinaceous casts and interstitial changes are more prevalent in the deeper regions of the cortex than in the superficial zone (14). Other workers reported increased susceptibility of juxtamedullary nephrons in the presence of proteinuria and hypertension (27). To gain some insight into the mechanism that may be involved in the discordant changes in whole GFR and SNGFR in AO rats, we
quantified the presence of proteinaceous casts in renal cortex and medulla. We demonstrated that proteinaceous casts resulting from AO occur in both cortex and medulla but are more severe in the medulla. PDTC treatment significantly prevented the formation of casts in both renal compartments. These findings suggest that obstruction of both medullary and cortical nephrons induced by AO results in a reduction in the whole kidney GFR and the increase in SNGFR in the remaining functional nephrons, sampled by micropuncture, represents a compensatory response to the loss of functional renal mass.

We observed that PDTC treatment to AO rats increased the $K_f$ by 46%. Although not statistically significant, this change may represent an effect mediated by PDTC. The regulation of $K_f$ is complex and may be modified when there are changes in hydraulic permeability (k) or in the surface area (A) of glomerular capillaries. While glomerular podocytes, endothelial and mesangial cells may contribute to changes in $K_f$, effective
GLOMERULAR HYPERTENSION DURING INTENSE PROTEINURIA

Table 2. Quantification of proteinaceous casts in renal cortex and medulla in saline, AO, and AO+PDTC groups evaluated at day 14

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>AO</th>
<th>AO+PDTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>3±2</td>
<td>61±12*</td>
<td>19±11†</td>
</tr>
<tr>
<td>Medulla</td>
<td>20±12</td>
<td>382±59*</td>
<td>33±25†</td>
</tr>
</tbody>
</table>

Data are means ± SD. Pixels/200 field. *P < 0.002 vs. saline. †P < 0.03 vs. AO. ‡P < 0.002 vs. AO.

glomerular filtration also requires that a semi-permeable glomerular basement membrane is maintained. For example, it is possible that PDTC may have resulted in less mesangial contraction, which could increase Kf by increasing capillary surface area (32). PDTC is capable of activating Akt-GSK signaling pathway and MAPK family, including ERK, SAPK/JNK, and p38 cascades in neuronal cells, cell lines PC12 and RAW 264.7 as well as in embryonic hippocampal progenitor cells (10, 22, 26, 40). Thus, given the complexity of Kf regulation, it is possible that PDTC might have had an effect on endogenous glomerular cells that may have resulted in increased Kf in AO PDTC-treated animals.

A striking finding in the present studies was the increase in afferent arteriolar thickness observed in AO rats. In previous studies, we documented that glomerular capillary hypertension is directly correlated with arteriolar remodeling (35) and is likely the consequence of defective afferent arteriolar regulation. Other investigators showed that neutrophil-induced acute oxidative stress is associated with glomerular hypertension resulting from afferent arteriolar vasoconstriction without systemic hypertension (51). In that study, the rise in glomerular pressure represented one of the first renal physiological changes before renal structural changes had occurred.

In the current study, both mechanisms appeared to be present. Thus, we found that PGC was significantly correlated with both an increase in the ER/AR ratio and with systemic SBP (ER/AR vs. Pgc, r = 0.74, P < 0.001; SBP vs. Pgc, r = 0.68, P = 0.001) consistent with both increased ER and systemic hypertension playing roles in causing glomerular hypertension in the AO rats.

Previously cited studies (51) reported that acute oxidative stress induced by polymorphonuclear cell infiltration was associated with a profound cortical vasoconstriction; in contrast, in our study we observed hyperfiltration in surface nephrons. This apparent discrepancy may be due to the fact that the previous studies were done after 18 h and ours after 2 wk of oxidative stress. Since oxidative stress is one of the first manifestations of proteinuria-induced renal damage (24), it is likely that AO rats might have developed glomerular hypertension and renal vasoconstriction early during the course of intense proteinuria as shown by Yoshioka and Ichikawa (51) and resulted in single-nephron hyperfiltration later, as shown in the present studies. In this setting, perpetuation of glomerular hypertension may be accounted by an afferent arteriole autoregulatory dysfunction induced by the preglomerular arteriolar remodeling as suggested by the significant correlation between M/L ratio and glomerular pressure (r = 0.77, P < 0.0001), as well as by the persistent proportional increment in effenter resistance. Exactly how the inflammation may cause the arteriopathy is not clear, but we observed this in a number of models (revised in Ref. 35). While a crosstalk between T NF-κB and afferent arteriolar remodeling is possible, it is also possible that the anti-inflammatory PDTC may be directly working on the arteriole (38).

In conclusion, we present the following concept for how glomerular injury develops in the AO model. In the AO model, there is evidence that intense proteinuria is associated with significant changes in glomerular podocytic structure that favors the traffic of proteins to tubular lumen (13). In turn, this could stimulate an acute increase in oxidative stress, enhanced by the infiltration of leukocytes, and associated with the stimulation of NF-κB by endogenous inflammatory cells. The acute rise in oxidative stress results in glomerular hypertension due to an effect on the efferent arteriole. Over time, however, there are afferent arteriolar structural changes that are mediated by the inflammation and oxidative stress that result in impaired autoregulation. The maintenance of glomerular hypertension also helps to maintain and accelerate the proteinuria, resulting in a vicious circle. Treatment with PDTC helps break this cycle of oxidative stress and inflammation and preserves the arteriolar morphology, thus leading to a reduction in glomerular pressure and reduction in proteinuria. Indeed, a positive correlation between proteinuria and glomerular pressure was noted (r = 0.64, P = 0.0003). Since reduction of glomerular pressure with PDTC significantly reduced, but did not completely suppress, urinary protein excretion, it is likely that other mechanisms participate in the alteration of the podocyte structure allowing the increased traffic of proteins in this model (23). Nevertheless, these studies support the role of anti-inflammatory agents in the preservation of afferent

Table 3. Activity of some antioxidant enzymes in renal cortex and medulla in saline, AO, and AO+PDTC groups evaluated at day 14

<table>
<thead>
<tr>
<th></th>
<th>CAT, k/mg Protein</th>
<th>GPX, U/mg Protein</th>
<th>GR, U/mg Protein</th>
<th>Total SOD, U/mg Protein</th>
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</thead>
<tbody>
<tr>
<td>Cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.321±0.055</td>
<td>0.260±0.025</td>
<td>0.052±0.002</td>
<td>17.6±1.8</td>
</tr>
<tr>
<td>AO</td>
<td>0.211±0.112*</td>
<td>0.180±0.082*</td>
<td>0.041±0.013</td>
<td>12.3±4.5</td>
</tr>
<tr>
<td>AO+PDTC</td>
<td>0.265±0.0190</td>
<td>0.286±0.025†</td>
<td>0.061±0.002†</td>
<td>16.0±2.4</td>
</tr>
<tr>
<td>Medulla</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.148±0.005</td>
<td>0.084±0.004</td>
<td>0.046±0.001</td>
<td>17.0±0.6</td>
</tr>
<tr>
<td>AO</td>
<td>0.046±0.02*</td>
<td>0.053±0.01*</td>
<td>0.037±0.004</td>
<td>5.6±1.8*</td>
</tr>
<tr>
<td>AO+PDTC</td>
<td>0.086±0.007*</td>
<td>0.092±0.004†</td>
<td>0.052±0.002†</td>
<td>9.9±0.71*</td>
</tr>
</tbody>
</table>

Data are means ± SD, n = 6–10. CAT, catalase; GPX, glutathione peroxidase; GR, glutathione reductase; SOD, superoxide dismutase. *P < 0.05 vs. saline. †P < 0.05 vs. AO.
arteriolar structure and function that may help preserve renal autoregulation and long-term renal function.

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