Advanced Oxidation Protein Products Accelerate Renal Fibrosis in a Remnant Kidney Model

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Accumulation of plasma advanced oxidation protein products (AOPP) has been found in patients with chronic kidney disease. However, the biologic consequences of AOPP consumption on progression of renal disease are still unclear. For testing of the hypothesis that AOPP accelerate progression of chronic kidney disease, Sprague-Dawley rats were subjected to five-sixths nephrectomy (5/6 Nx) or to sham operation. Rats in each group were randomly assigned in three subgroups (n = 30 in each group) and treated with repeated intravenous injections of AOPP-modified rat serum albumin (RSA), unmodified RSA, or vehicle for indicated period. Compared with RSA- or vehicle-treated 5/6 Nx rats, AOPP RSA–treated 5/6 Nx rats displayed greater proteinuria, higher serum creatinine, and lower creatinine clearance. AOPP challenge resulted in more renal hypertrophy, higher macrophage influx, and greater renal fibrosis in the remnant kidney. Chronic administration of AOPP in sham-operated rats increased urinary protein excretion and renal macrophage infiltration, but histologic renal fibrosis was not observed during the study period. AOPP treatment enhanced AOPP level in renal tissue. This was associated with marked increase of thiobarbituric acid reactive substances, decrease of glutathione peroxidase activity, and upregulated expression of monocyte chemoattractant protein-1 and TGF-β1 in renal cortex. These data indicate that AOPP might be a new and potentially important mediator of renal fibrosis in the remnant kidney. Chronic accumulation of AOPP promotes renal fibrosis probably via a redox-sensitive inflammatory pathway.

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C hronic kidney disease (CKD) is a worldwide health problem, and the number of patients with CKD is increasing rapidly (1). Progressive renal disease is characterized by the development of glomerulosclerosis and interstitial fibrosis. This final common pathway predicts the degree of renal dysfunction and long-term prognosis for almost all forms of CKD. Therefore, exploring the factors that may promote the process would be important for developing new strategies to suppress the shift to this common pathway.

The factors that have been implicated in the initiation of this cascade are numerous and include angiotensin II, growth factors, cytokines, oxygen metabolites, and mechanical factors (2). Recent studies found that plasma concentration of advanced oxidation protein products (AOPP) significantly increased with the progression of renal dysfunction in patients with a variety of CKD (3), as well as in patients with diabetes (4). Previous study revealed that AOPP were carried by plasma proteins, especially albumin (3). AOPP formed in vitro by exposure of serum albumin to hypochlorous acid (HOCl). In vivo, plasma concentration of AOPP closely correlated with levels of dityrosine, a hallmark of oxidized protein, and pentosidine, a marker of protein glycoxidation that is tightly related to oxidative stress (5). Thus, AOPP might be formed during oxidative stress by reaction of plasma proteins with chlorinated oxidants and have been considered as novel markers of oxidant-mediated protein damage (3).

Much recent interest has focused on the role of AOPP in atherosclerosis. Plasma AOPP are closely correlated to carotid intima media thickness (6) and atherosclerotic cardiovascular events (7) in ESRD. Chronic administration of AOPP accelerates atherosclerosis in a hyperlipidemic rabbit model (8). A clinical study revealed a close relationship between AOPP levels and serum markers of monocyte activation (9). These data suggest that these oxidized proteins by themselves may contribute to the inflammatory process that is associated with CKD. A recent study that was performed in a large cohort of patients with IgA nephropathy suggested that plasma AOPP level was predictive of progressive renal disease (10). However, there is little evidence that AOPP contribute to progression of renal disease. Our study was conducted to test the hypothesis that AOPP accelerate renal fibrosis and contribute to progression of CKD.

Materials and Methods

AOPP Rat Serum Albumin Preparation and Determination

AOPP rat serum albumin (RSA) was prepared in vitro as described previously (3,8). Briefly, RSA (100 mg/ml; Sigma, St. Louis, MO) was
exposed to HOCl (200 mmol/L; Fluke, Buchs, Switzerland) for 30 min at room temperature and then dialyzed overnight against PBS to remove any free HOCl. Prepared samples were passed through a Detoxi-Gel column (Pierce, Rockford, IL) to remove any contaminating endotoxin. Endotoxin levels in the preparation were tested with Limulus Amoeboocyte Lysate kit (Sigma) and were found to be <0.025 EU/ml. AOPP content in the preparation was determined as described previously (3,8). Briefly, 200 μl of prepared sample or chloramine-T (standard curve; Sigma) was placed in a 96-well plate (Corning Costar, New York, NY) and mixed with 20 μl of acetic acid. The absorbance of the reaction mixture at 340 nm was read immediately in a microplate reader (Wallac 1420, PerkinElmer, Turku, Finland). The content of AOPP in the AOPP RSA preparation was 5.03 ± 0.5 versus 0.3 ± 0.03 nmol/mg protein in unmodified RSA.

Plasma levels of AOPP were determined as described previously. For exclusion of the interference of turbidity of lipids on light absorption, samples were diluted 1:5 in PBS and centrifuged (10,000 × g, 1 h, 4°C). The samples below the lipid layer were used for AOPP measurement. AOPP in homogenized renal tissue were quantified by using spectrophotometry as described previously (11).

**Experimental Protocol**

All animal procedures were approved by the Animal Experiment Committee of Southern Medical University. Male Sprague-Dawley rats (initial weight 220 to 240 g; Southern Medical University Animal Experiment Center) were maintained under standardized conditions and fed a standard rodent diet that contained 16% protein. The rats were treated according to the 2×3×3 factorial design. Briefly, the rats were subjected either to five-sixths nephrectomy (5/6 Nx; n = 90) by performing a right nephrectomy with surgical resection of two thirds of the left kidney) or to sham operation (controls; n = 90). One week after the operation, the 5/6 Nx rats were randomized by the percent remnant kidney weight removed ([right kidney weight – weight of two poles of left kidney]/right kidney weight × 100) and were divided into three subgroups (groups 1 through 3; n = 30 in each group). The control rats also were randomly assigned to three subgroups (groups 4 through 6; n = 15 in each group). Rats then received the agents intravenously once every other day as follows: (1) AOPP RSA (20 mg/kg, groups 1 and 4), (2) unmodified RSA (20 mg/kg, groups 2 and 5), or (3) vehicle (PBS, groups 3 and 6) for indicated time. The dosage of AOPP and the injection interval were based on our preliminary studies indicating that by this procedure, plasma AOPP concentration increased by one-fold in sham-operated rats and by three-fold in 5/6 Nx rats compared with that in normal rats (12). At the end of 5, 9, and 13 wk after operation, the rats (n = 10 in each group at each time point) were anesthetized with sodium pentobarbital and exsanguinated. The left kidneys were collected after perfusion with 50 ml of ice-cold normal saline. The 24-h urine samples were collected in metabolic cages at end of the study period.

### Renal Function and BP

Serum and urine creatinine levels were determined using commercial kits (sarcosine oxidase-peroxidase-antiperoxidase; Zixing, Shanghai, China). The creatinine clearance (Ccr) was calculated as described previously and factored for body weight (13). Twenty-four-hour urinary protein excretion was measured using the Coomassie Blue method (14).

For BP measurement, PE-50 catheters were inserted into the femoral artery before the rats were killed. Systolic BP (SBP) was measured by a pressure transducer (Gould, Eichstetten, MA) that was connected to a physiologic recorder (Gilion Medical Electronics, Middleton, OH).

### Renal Morphologic Analyses

Tissue for light microscopy was fixed in 10% phosphate-buffered formalin and embedded in paraffin. Four-micrometer-thick sections were processed for periodic acid-Schiff and Masson’s trichrome staining. Morphologic analyses were performed by an experienced pathol-

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**Table 1. Left kidney weight/body weight and glomerular volume**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Week 5</th>
<th>Week 9</th>
<th>Week 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>kidney weight/body weight (g/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group 1 (5/6 Nx + AOPP)</td>
<td>4.37 ± 0.45&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.65 ± 0.65&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>4.71 ± 0.38&lt;sup&gt;d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 2 (5/6 Nx + RSA)</td>
<td>4.10 ± 0.61&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.13 ± 0.42&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.23 ± 0.30&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 3 (5/6 Nx + vehicle)</td>
<td>4.08 ± 0.53&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.05 ± 0.58&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.25 ± 0.35&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 4 (control + AOPP)</td>
<td>3.41 ± 0.27</td>
<td>3.43 ± 0.20</td>
<td>3.49 ± 0.17</td>
</tr>
<tr>
<td>group 5 (control + RSA)</td>
<td>3.20 ± 0.25</td>
<td>3.18 ± 0.41</td>
<td>3.26 ± 0.25</td>
</tr>
<tr>
<td>group 6 (control + vehicle)</td>
<td>3.23 ± 0.32</td>
<td>3.22 ± 0.29</td>
<td>3.28 ± 0.36</td>
</tr>
<tr>
<td>Glomerular volume (10&lt;sup&gt;6&lt;/sup&gt; μm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group 1 (5/6 Nx + AOPP)</td>
<td>1.84 ± 0.16&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>2.31 ± 0.17&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>2.34 ± 0.17&lt;sup&gt;d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 2 (5/6 Nx + RSA)</td>
<td>1.77 ± 0.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.04 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.13 ± 0.14&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 3 (5/6 Nx + vehicle)</td>
<td>1.78 ± 0.12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.11 ± 0.18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.17 ± 0.13&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 4 (control + AOPP)</td>
<td>1.03 ± 0.07</td>
<td>1.13 ± 0.07&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.22 ± 0.08&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 5 (control + RSA)</td>
<td>1.01 ± 0.06</td>
<td>1.05 ± 0.06</td>
<td>1.07 ± 0.09</td>
</tr>
<tr>
<td>group 6 (control + vehicle)</td>
<td>1.02 ± 0.05</td>
<td>1.06 ± 0.04</td>
<td>1.09 ± 0.10</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are means ± SD, n = 10 in each group at each time point. 5/6 Nx, five-sixths nephrectomy; AOPP, advanced oxidation protein products; RSA, rat serum albumin.

<sup>b</sup>Factorial analysis, model style, F = 291.396, P < 0.001; different treatment, F = 14.249, P < 0.001.

<sup>c</sup>Factorial analysis, model style, F = 3659.886, P < 0.001; different treatment, F = 25.170, P < 0.001; different time, F = 94.203, P < 0.001; model style × treatment, F = 3.168, P = 0.045, time × treatment, F = 2.780, P = 0.029.

<sup>d</sup>P < 0.01 versus controls with the same treatment.

<sup>e</sup>P < 0.05 versus RSA- and vehicle-treated groups that received the same operation.
ogist who was blinded to the source of the tissue. The extent of glomerular sclerosis was assessed by a semiquantitative analysis as described by Raij et al. (15). At least 50 glomeruli from each kidney were graded on the periodic acid-Schiff–stained sections according to the following criteria: 0, no sclerosis; 1, 25% cross-sectional sclerosis; 2, 25 to 50% exhibiting sclerosis; 3, 50 to 75% exhibiting sclerosis; and 4, 75% cross-sectional sclerosis. The sclerosis index for each rat was calculated as follows: \( \frac{N_1}{n} + \frac{N_2}{n} + \frac{N_3}{n} + \frac{N_4}{n} \), where \( N_1, N_2, N_3, \) and \( N_4 \) represent the numbers of glomeruli that exhibited grades 1, 2, 3, and 4, respectively, and \( n \) represents the number of glomeruli assessed (16). Similarly, trichrome-stained sections from each kidney were graded for the presence of interstitial fibrosis according to the following scale: 0, no evidence of interstitial fibrosis; 1, 25% involvement; 2, 25 to 50% involvement; and 3, 50% involvement (17).

The scale for each rat was reported as the mean of 20 random high-power \((\times 400)\) fields per section. The average glomerular tuft volume was estimated at each time of death using the method described previously (18). The mean glomerular cross-sectional area \((A_g)\) was determined by averaging approximately 50 glomerular sections. Individual glomerular volume \((V_g)\) was calculated as \( V_g = 1.25 \times (A_g)^{3/2} \) (18).

Renal Immunohistochemical Analyses

The immunoperoxidase staining was performed as described previously (19), with the following antibodies: Macrophage infiltration was detected with monoclonal anti–ED-1 (Serotec, Oxford, UK), TGF-β1 expression was detected with polyclonal rabbit anti-rat TGF-β1 (Santa Cruz Biotechnology, Santa Cruz, CA), monocyte chemoattractant protein-1 (MCP-1) was analyzed with polyclonal rabbit anti-rat MCP-1 (Boster Biologic, Wuhan, China), and α-smooth muscle actin (α-SMA) was analyzed with monoclonal anti–α-SMA (Boster Biologic). Control experiments included omission of the primary antibodies and substitution of the primary antibodies with nonimmune rabbit or mouse IgG. Macrophage infiltration was quantified by counting the number of ED-1-positive cells in 30 glomerular profiles and in 20 randomly chosen 0.25\(\times\)0.25-mm areas of tubulointerstitium for each kidney (20). The intensity of glomerular staining of TGF-β1, MCP-1, and α-SMA was evaluated under \(\times 400\) magnification according to the following scale: 0, no staining; 1, weak and spotty intraglomerular staining; 2, moderate and segmental intraglomerular staining; and 3, strong and diffuse (involving >50%) intraglomerular staining. Likewise, the intensity of tubulointerstitial staining in cortical areas was assessed under \(\times 250\) magnification as the following scale: 0, no staining; 1, <25% involvement; 2, 25 to 50% involvement; and 3, >50% involvement. All but at least 20 glomeruli and 20 randomly selected cortical tubulointerstitial areas from each sample were evaluated.

Reverse Transcriptase–PCR

Total RNA was extracted from renal cortex tissues using RNeasy mini kit (Qiagen, Valencia, CA). Primers for MCP-1, TGF-β1, and...
glyceraldehyde-3-phosphate dehydrogenase were designed and synthesized on the basis of published sequence of these genes (20). The upstream and downstream of these primers are as follows: (1) MCP-1 5'-GTC ACC AAG CTC AAG AGA GAG A-3' and 5'-GAG TGG ATG CAT TAG CCT CAG A-3', (2) TGF-β1 5'-CTT CAG CTC CAC AGA GAA GAA CTG C-3' and 5'-CAC GAT CAT GTT GGA CAA CTG CTC C-3', and (3) glyceraldehyde-3-phosphate dehydrogenase 5'-AAT GCA TCC TGC ACC ACC AA-3' and 5'-GTA GCC ATA TTC ATT GTA-3'. Reverse transcriptase–PCR was performed using Qiagen One Step reverse transcriptase–PCR kit according to the manufacturer's instructions (Qiagen). PCR products that were resolved in 2% agarose gels were photographed under ultraviolet light. Densities of bands were measured by scanning densitometry with UVIBAND V.99 Software (UVI, SJ, Cambridge, England).

Parameters of Oxidative Stress and Inflammation

Lipid peroxides in the renal cortical homogenate were measured as thiobarbituric acid reactive substances (TBARS) by fluorometric assay (21). Activity of glutathione peroxidase (GSHPx) in the renal tissues was measured as described previously (22). Urinary MCP-1 and TGF-β1 were quantified by ELISA using commercial kits according to the manufacturer's instruction (BioSource, Camarillo, CA).

Statistical Analyses

All data are presented as means ± SD. Main and interactive effect of AOPP and 5/6 Nx were analyzed by factorial analysis. Serum creatinine (Scr), Ccr, and urinary protein excretion were adjusted by covariates before intervention. Differences in the variables between groups were determined by one-way ANOVA followed by LSD method when \( P \leq 0.05 \). Differences in the variables at each time point between 5/6 Nx and sham-operated rats with the same treatment were compared by independent samples \( t \) test. The relationship between variables was assessed by Pearson correlation analysis. Statistical analyses were conducted with SPSS 12.0 for Windows (SPSS, Chicago, IL). Significance was defined as \( P \leq 0.05 \).

Results

Effect of AOPP Administration on Renal Compensatory Growth

All rats survived. The weight of the remnant kidney increased with time in 5/6 Nx rats. The increment, expressed as the remnant kidney weight/body weight, was relatively higher in the AOPP RSA–treated rats than in unmodified RSA- or vehicle-treated rats (Table 1). Similarly, glomerular volume in the remnant kidney increased with time, and the increment was
expressed essentially in vascular smooth muscle cells. Positive
the score was much lower in sham-operated than in 5/6 Nx rats
5/6 Nx rats. AOPP administration significantly increased
AOPP-challenged sham-operated controls at week 13. In con-
three-fold in AOPP-treated 5/6 Nx rats and by one-fold in
concentration of plasma AOPP increased by approximately
and control rats. Compared with vehicle-treated controls, the
levels in plasma and renal tissue in both 5/6 Nx
levels in the renal homogenates also increased in 5/6 Nx rats
administration significantly increased the TBARS level from
administration increased glomerular volume from week 9 as
compared with RSA- or vehicle-treated rats, but there was no
significant difference in kidney weight/body weight among
subgroups.
In sham-operated RSA- and vehicle-treated rats, α-SMA was
expressed essentially in vascular smooth muscle cells. Positive
α-SMA staining of glomeruli and interstitium was observed in
5/6 Nx rats. AOPP administration significantly increased
α-SMA staining score in both 5/6 Nx and control rats, although
the score was much lower in sham-operated than in 5/6 Nx rats
(Figure 1).

Effect of AOPP Administration on Renal Tissue Damage
Glomerulosclerosis and interstitial fibrosis progressed with
time in 5/6 Nx rats. Compared with RSA- and vehicle-treated
rats, AOPP RSA–treated rats showed significantly increased
glomerulosclerosis index (Figure 2A) and interstitial fibrosis
score from week 5 (Figure 2B). No significant histologic character-
istic of fibrosis could be detected in rats that underwent
sham operation.
Few ED-1–positive cells in normal renal tissue were removed
at the time of the surgery and in sham-operated rats that were
were treated with RSA or vehicle. Macrophage infiltration was
evident in both glomeruli and interstitium of the remnant kidney.
At 5 wk after surgery, the number of infiltrated macrophages
was increased markedly in AOPP-challenged versus RSA- or
vehicle-treated 5/6 Nx rats. These increases were sustained at
later time points (Figure 2, C and D). Similarly, AOPP admin-
istration resulted in macrophage infiltration in sham-operated
controls (Figure 2, C and D).

Effect of AOPP Administration on Renal Function and BP
Subtotal nephrectomy resulted in significant renal dysfunc-
tion, as evidenced by progressive increase in Scr (Figure 1C)
and decline of Ccr (Figure 1D). Urinary protein excretion (Fig-
ure 1E) increased significantly in 5/6 Nx rats. SBP did not
change during the first 5 wk but rose progressively at later time
points (Figure 1F). AOPP administration in 5/6 Nx rats signif-
icantly worsened renal dysfunction and proteinuria without
altering SBP (Figure 1). AOPP challenge also increased urinary
protein excretion in sham-operated rats. However, there was no
statistical difference in Scr, Ccr, and SBP between AOPP-treated
and RSA- or vehicle-treated controls (Figure 1).

Effect of AOPP Administration on Renal Redox Reaction
Plasma AOPP levels increased by two-fold in RSA- and
vehicle-treated 5/6 Nx rats compared with RSA- and vehicle-
treated sham-operated controls (P < 0.001; Figure 3A). AOPP
levels in the renal homogenates also increased in 5/6 Nx rats
(Figure 3B). Chronic administration of AOPP significantly in-
creased AOPP levels in plasma and renal tissue in both 5/6 Nx
and control rats. Compared with vehicle-treated controls, the
concentration of plasma AOPP increased by approximately
three-fold in AOPP-treated 5/6 Nx rats and by one-fold in
AOPP-challenged sham-operated controls at week 13. In con-
trast, there was no significant difference in plasma albumin
levels among groups at any time point (data not shown).
Plasma AOPP level correlated significantly with glomeruloscle-
rosis index (r = 0.782, n = 180, P < 0.001), tubulointerstitial
scores (r = 0.817, n = 180, P < 0.001), and the number of
macrophages in both glomeruli (r = 0.845, n = 180, P < 0.001)
and interstitium (r = 0.841, n = 180, P < 0.001). Likewise, there
was a close correlation between plasma AOPP level and urin-
ary protein excretion (r = 0.807, n = 180, P < 0.001) or Ccr (r =
−0.730, n = 180, P < 0.001).

As shown in Table 2, the TBARS levels in renal cortical
homogenates increased significantly in 5/6 Nx rats compared
with RSA- or vehicle-treated sham-operated controls. AOPP
administration significantly increased the TBARS level from
week 5 in both 5/6 Nx and sham-operated rats as compared
with their respective RSA- or vehicle-treated controls. GSHPx
activity decreased by 40% in renal homogenates in 5/6 Nx rats
at week 5. At week 13, GSHPx activity tended to restore in RSA-
and vehicle-treated rats but remained decreased in AOPP-
treated rats. There was a close relationship between renal
Table 2. Parameters of renal redox reaction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Week 5</th>
<th>Week 9</th>
<th>Week 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS levels (nmol/mg protein)b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group 1 (5/6 Nx + AOPP)</td>
<td>4.13 ± 0.96&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>5.06 ± 0.99&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>7.40 ± 1.45&lt;sup&gt;d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 2 (5/6 Nx + RSA)</td>
<td>3.09 ± 0.91&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.24 ± 0.55&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.91 ± 1.38&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 3 (5/6 Nx + vehicle)</td>
<td>3.12 ± 0.51&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.15 ± 0.72&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.65 ± 1.15&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 4 (control + AOPP)</td>
<td>2.52 ± 0.57&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2.97 ± 0.63&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.17 ± 0.97&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 5 (control + RSA)</td>
<td>1.69 ± 0.54</td>
<td>1.64 ± 0.49</td>
<td>1.71 ± 0.24</td>
</tr>
<tr>
<td>group 6 (control + vehicle)</td>
<td>1.53 ± 0.47</td>
<td>1.59 ± 0.36</td>
<td>1.63 ± 0.56</td>
</tr>
<tr>
<td>GSHPx activity (U/mg protein)&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group 1 (5/6 Nx + AOPP)</td>
<td>1.62 ± 0.29&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>1.11 ± 0.17&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>1.24 ± 0.20&lt;sup&gt;d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 2 (5/6 Nx + RSA)</td>
<td>2.78 ± 0.62&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.36 ± 0.66&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.27 ± 0.83</td>
</tr>
<tr>
<td>group 3 (5/6Nx + vehicle)</td>
<td>2.94 ± 0.60&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.79 ± 0.80&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.19 ± 0.89</td>
</tr>
<tr>
<td>group 4 (control + AOPP)</td>
<td>3.51 ± 0.44&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.73 ± 0.39&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.39 ± 0.70&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>group 5 (control + RSA)</td>
<td>4.63 ± 0.97</td>
<td>4.71 ± 0.93</td>
<td>4.77 ± 0.83</td>
</tr>
<tr>
<td>group 6 (control + vehicle)</td>
<td>4.73 ± 0.98</td>
<td>4.77 ± 0.95</td>
<td>4.69 ± 0.92</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are means ± SD, n = 10 in each group at each time point. GSHPx, glutathione peroxidase; TBARS, thiobarbituric acid reactive substances.

<sup>b</sup>Factorial analysis, model style, F = 412.456, P < 0.001; different treatment, F = 57.082, P < 0.001; different time, F = 63.119, P < 0.001.

<sup>c</sup>Factorial analysis, model style, F = 257.887, P < 0.001; different treatment, F = 90.105, P < 0.001; different time, F = 8.164, P < 0.001; model style × treatment, F = 5.141, P < 0.001; time × treatment, F = 3.950, P = 0.004

<sup>d</sup>P < 0.05 versus controls with the same treatment.

<sup>e</sup>P < 0.05 versus RSA- and vehicle-treated groups that received the same operation.

AOPP and TBARS levels (r = 0.841, n = 180, P < 0.001) or GSHPx activity (r = -0.520, n = 180, P < 0.001).

Effect of AOPP Administration on MCP-1 and TGF-β1 Expression

In 5/6Nx rats, the levels of urinary MCP-1 (Figure 4A) and TGF-β1 (Figure 5A) were significantly higher than that in sham-operated controls. AOPP treatment significantly increased urinary MCP-1 and TGF-β1 excretion as compared with RSA- or vehicle-treated subgroups. Chronic AOPP loading in sham-operated rats also increased urinary MCP-1 and TGF-β1 levels, but the increment was much lower than that in 5/6Nx rats.

Immunohistological studies showed that MCP-1 and TGF-β1 staining was limited to minimal positive reaction of tubular cells and negative in glomeruli. Among 5/6Nx rats, positive staining of MCP-1 (Figure 4, C through E) and TGF-β1 (Figure 5, C through E) was observed in both tubulointerstitium and glomeruli. Higher staining scores were found in AOPP- compared with RSA- or vehicle-treated 5/6Nx rats (Figures 4E and 5E). Positive MCP-1 and TGF-β1 staining also could be found in glomeruli in sham-operated controls that were challenged by AOPP, but there was no significant difference in staining score for TGF-β1 in tubulointerstitium between AOPP- and RSA- or vehicle-treated controls (Figure 5, C and D).

Expression of MCP-1 (Figure 4B) and TGF-β1 mRNA (Figure 5B) was upregulated in renal cortex of 5/6Nx rats as compared with sham-operated controls. AOPP administration significantly increased MCP-1 and TGF-β1 mRNA expression in both 5/6Nx and sham-operated rats.

Discussion

Our study demonstrated that elevation of plasma AOPP level in the remnant kidney model resulted in accelerated progression of renal damage, as evidenced by marked increase of tubular fibrosis and glomerulosclerosis, worsened proteinuria, and deteriorated renal dysfunction. The renal pathogenic role of these oxidized proteins was supported further by the finding that AOPP administration increased the renal protein excretion in sham-operated rats, although renal fibrosis and dysfunction were not observed during the period of the study. AOPP RSA but not unmodified RSA or vehicle promoted progression of CKD, suggesting that the aggravating effect was due to AOPP and not a property of RSA or other contaminants. To our knowledge, this is the first study to provide in vivo evidence for a causal role of chronic AOPP accumulation on progressive CKD.

The mechanisms by which AOPP accelerate renal fibrosis remain to be clarified. In our study, the glomerular volume and the remnant kidney weight were higher in AOPP RSA–treated than in RSA- or vehicle-treated 5/6Nx rats, and α-SMA expression, a cytoskeletal marker of myofibroblasts, significantly increased in the AOPP-treated remnant kidney model. Furthermore, the subsequent renal fibrosis was accelerated significantly by AOPP administration. These results suggest that AOPP might be involved in the pathogenesis of renal mass hypertrophy and remodeling, as well as the subsequent fibrogenic process in the remnant kidney. This observation supports the previous hypothesis that the hypertrophy of remnant nephrons and the progression of glomerular sclerosis might be
Figure 4. AOPP administration increased urinary monocyte chemoattractant protein-1 (MCP-1) excretion and upregulated renal expression of MCP-1. Rats were treated as described in Figure 1. (A) Urinary MCP-1 levels. (B) Representative gel profile of MCP-1 mRNA by reverse transcriptase–PCR (RT-PCR) and the quantitative results of the band densities at week 13. (C) Immunohistochemical staining score for MCP-1 in glomeruli. (D) Immunohistochemical staining score for MCP-1 in tubulointerstitium. (E) Representative immunohistochemical staining profiles of MCP-1 expression at week 13. □, group 1; ▪, group 2; □, group 3; ▪, group 4; ▪, group 5; □, group 6. Data are means ± SD; n = 10 in each group at each time point. *P < 0.05 versus sham-operated rats with the same treatment; *P < 0.05 versus RSA- and vehicle-treated groups that received the same operation.
Figure 5. AOPP administration increased urinary TGF-β1 excretion and upregulated renal expression of TGF-β1. Rats were treated as described in Figure 1. (A) Urinary TGF-β1 levels. (B) Representative gel profiles of TGF-β1 mRNA by RT-PCR and the quantitative results of the band densities at week 13. (C) Immunohistochemical staining score for TGF-β1 in glomeruli. (D) Immunohistochemical staining score for TGF-β1 in tubulointerstitium. (E) Representative immunohistochemical staining profiles of TGF-β1 expression at week 13. □, group 1; ■, group 2; □, group 3; ■, group 4; ■, group 5; ■, group 6. Data are means ± SD; n = 10 in each group at each time point. *P < 0.05 versus sham-operated rats with the same treatment; *P < 0.05 versus RSA- and vehicle-treated groups that received the same operation.
related to some circulating substances that accumulated with renal mass reduction (23,24). The remnant kidney model that was used in our study was prepared by a uninephrectomy followed by an ablation of two thirds of the contralateral kidney. Consistent with the previous studies (25–27), the BP in this excision model did not increase during the first weeks, contrasting with the model of renal artery branch ligation. Moreover, AOPP administration did not alter significantly the BP even in the later period of renal mass ablation, suggesting that the aggravating effect of AOPP might not be attributed to increase of systemic BP. Another possibility is that, being a prooxidant, exogenously administered HOCl might be responsible for the renal damage. However, the AOPP that were used in the study were dialyzed thoroughly before administration to remove any free HOCl. It seems unlikely that the pathobiologic effects of the HOCl-modified protein are due to the HOCl itself.

Chronic renal insufficiency is associated with oxidative stress that is characterized by increased generation of reactive oxygen species and decreased activity of antioxidant system (28,29). Oxidative stress has emerged as a potential mechanism that is involved in the progression of CKD (30–32) and contributed to hypertrophy of tubular cells as a result of their impact on cell-cycle regulation (33,34). The importance of oxidative stress is illustrated further by the use of antioxidants to antagonize the proteinuria and glomerulosclerosis in the remnant kidney model (35). However, although the experimental evidence supports a role for oxidative stress in the pathogenesis of CKD, little is known about the underlying mechanisms (28,29). In our results, AOPP levels in plasma and renal tissue increased after subtotal nephrectomy, suggesting that, as reported previously, AOPP spontaneously generated after renal mass reduction. Chronic administration of AOPP in this remnant kidney model significantly increased the renal levels of AOPP, which were accompanied by increased levels of TBARS and reduced GSHPx activity. Moreover, AOPP administration induced imbalance of redox reaction in rats with intact kidney and normal renal function, suggesting that the worse of oxidative stress could not attribute to progression of renal dysfunction alone. Taken together, these results suggest that AOPP might be potential inducers of oxidative stress in CKD. The close relationship between AOPP and TBARS levels or GSHPx activity and the in vitro studies demonstrating the respiratory burst of human neutrophils that were exposed to AOPP (36) provide further evidence to support the notion. Therefore, it seems reasonable to assume that chronic AOPP accumulation, such as in CKD, may constitute a new molecular basis for enhanced oxidative stress that plays a potential role in disease progression.

Reactive oxygen species that are generated in oxidative stress have been demonstrated to be signals for the activation of nuclear factor-κB, the major inflammatory transcription factor (37). Our data provide three lines of in vivo evidence for the proinflammatory effects of AOPP in renal tissue. First, chronic AOPP challenge increased macrophage infiltration in both remnant and normal kidney. Although the precise mechanism whereby inflammatory cells cause fibrosis is uncertain, most reports suggest that macrophages infiltration promotes renal fibrosis (38–40). Second, AOPP administration upregulated the expression of proinflammatory chemokine MCP-1. Overexpression of MCP-1 has been found to be associated with the cellular inflammation and the myofibroblastic activity in renal parenchyma (41,42). Third, chronic AOPP administration increased expression of TGF-β1, a well-documented fibrogenic growth factor that plays a major role in the pathogenesis of renal inflammation and fibrosis (43,44). Further support for the proinflammatory effects of HOCl-modified protein comes from an in vitro study that demonstrated that HOCl-modified LDL upregulates inflammatory and fibrogenic genes in human proximal tubular epithelial cells (45). Taken together, our findings provide new in vivo evidence to suggest that AOPP may act as a novel class of proinflammatory mediators. Given that AOPP accumulation occurs from an early stage of CKD, when Ccr remains at approximately 41 to 80 ml/min (9), it is plausible to propose that AOPP accumulation followed by reduction of renal mass can increase oxidative stress and inflammation and that enhanced oxidative stress and inflammation may further increase AOPP formation through stimulation of leukocytes to produce more oxidants (45). This positive feedback loop could amplify or maintain the imbalance of redox reaction and inflammatory status and thereby promote the renal fibrosis. If this hypothesis is true, then inhibition of AOPP generation or blocking the pathobiologic effect of AOPP might ameliorate the progression of CKD.

Conclusion

We have identified AOPP as a new class and potentially important mediators of renal progression in the remnant kidney model. AOPP accumulation promotes renal fibrosis and deteriorates renal dysfunction, probably via a redox-sensitive inflammatory pathway. Although one must be cautious in the interpretation of animal models to human disease, these studies provide a mechanism to explain recent clinical data (10) showing that AOPP is an independent predictor of progression of CKD.

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Disclosures

None.

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