Increased Oxidative Stress in Experimental Renovascular Hypertension

Lilach O. Lerman, Karl A. Nath, Martin Rodriguez-Porcel, James D. Krier, Robert S. Schwartz, Claudio Napoli, J. Carlos Romero

Abstract—The pathophysiological mechanisms responsible for maintenance of chronic renovascular hypertension remain undefined. Excess angiotensin II generation may lead to release of reactive oxygen species and increased vasoconstrictor activity. To examine the potential involvement of oxidation-sensitive mechanisms in the pathophysiology of renovascular hypertension, blood samples were collected and renal blood flow measured with electron-beam computed tomography in pigs 5 and 10 weeks after induction of unilateral renal artery stenosis (n=7) or sham operation (n=7). Five weeks after procedure, plasma renin activity and mean arterial pressure were elevated in hypertensive pigs. Levels of prostaglandin F2α (PGF2α)-isoprostanes, vasoconstrictors and markers of oxidative stress, also were significantly increased (157±21 versus 99±16 pg/mL; P<0.05) and correlated with both plasma renin activity (r=0.83) and arterial pressure (r=0.82). By 10 weeks, plasma renin activity returned to baseline but arterial pressure remained elevated (144±10 versus 115±5 mm Hg; P<0.05). Isoprostane levels remained high and still correlated directly with the increase in arterial pressure (r=0.7) but not with plasma renin activity. Stenotic kidney blood flow was decreased at both studies. In shock-frozen cortical tissue, ex vivo endogenous intracellular radical scavengers were significantly decreased in both kidneys. The present study demonstrates, for the first time, that in early renovascular hypertension, an increase in plasma renin activity and arterial pressure is associated with increased systemic oxidative stress. When plasma renin activity later declines, PGF2α-isoprostanes remain elevated, possibly due to local activation or slow responses to angiotensin II, and may participate in sustenance of arterial pressure. Moreover, oxidation-sensitive mechanisms may influence ischemic and hypertensive parenchymal renal injury. (Hypertension. 2001;37[part 2]:541-546.)

Key Words: hypertension, renal $\bullet$ angiotensin II $\bullet$ stress, oxidative $\bullet$ isoprostanes $\bullet$ renin

Renal artery stenosis is a major cause of renovascular hypertension (RVH) and may lead to ischemic nephropathy and end-stage renal disease. The role of the renal vasculature in eliciting RVH had been established as early as 1934, when Goldblatt et al demonstrated that partial obstruction of the renal artery increased mean arterial pressure (MAP). Nevertheless, to date, the mechanism responsible for maintenance of chronic RVH remains undefined.2

Involvement of the renin-angiotensin system in pathogenesis of this disorder has been well established but is puzzling given the near-normal circulating levels of angiotensin II (Ang II) often observed in chronic RVH.3 Researchers have speculated that RVH may be maintained by enhanced vascular responsiveness to the slow pressor effect of Ang II.4 This phenomenon is characterized by a gradual increase of MAP that can be induced by chronic systemic infusion of Ang II (over a period of 3 to 5 days) in subpressor doses, which are too low either to significantly increase its circulating levels or to evoke an immediate increase in MAP.5 We recently showed6,7 that development of slow responses to Ang II (during chronic systemic infusion of low-dose Ang II) was accompanied by an Ang II–induced increase of one of the systemic oxidative stress markers, prostaglandin F2α (PGF2α)–isoprostanes, the potential effects of which include a decrease in renal blood flow (RBF), sodium retention, and vasoconstriction.8,9 Such effects not only could play a role in development of slow responses to Ang II, but also could mediate and be more directly linked with sustenance of RVH than circulating Ang II levels. Because of ischemia and high intrarenal Ang II levels,10 the stenotic kidney conceivably could be a source of such mediators.

Nevertheless, the involvement of oxidation-sensitive mechanisms in the pathophysiology of RVH rather than exogenous Ang II infusion has not been fully explored.2,7 Thus, the present study was designed to examine the hypothesis that unilateral renal artery stenosis increased activation of oxidation-sensitive mechanisms in systemic circulation and stenotic kidney.

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Methods

Fourteen domestic female pigs were studied at baseline and 5 and 10 to 12 weeks after induction of renal artery stenosis (group 1; n=7), or after sham operation (group 2; n=7). In each study, systemic and renal vein blood samples were collected for measurement of PGF$_{2\alpha}$-isoprostanes and plasma renin activity (PRA), degree of stenosis was determined offline from the images by use of a standard quantitative coronary angiography system through assessment of the decrease in luminal diameter compared with a stenosis-free segment.12 On completion of each EBCT (“acute”) study, all catheters were removed, and the access vessels ligated.

Blood Pressure Measurement

Continuous (“chronic”) blood pressure recording was obtained by use of a PhysioTel telemetry system (Data Sciences) implanted at baseline in the left carotid artery after catheter withdrawal as previously described.13 MAP was recorded at 5-minute intervals and averaged for each 24-hour period. Levels reported (Table 1) were those obtained for 2 days before each study.

After completion of the series of experiments, pigs were allowed to recover and were euthanatized 2 to 4 days later (to ensure complete renal recovery and contrast clearance). Kidneys were dissected, immediately sliced into sections, shock-frozen in liquid nitrogen, and maintained at −80°C.

EBCT Image Analysis

EBCT images were reconstructed by use of a standard tomographic algorithm and displayed on a Sun System computer workstation with the Analyze software package (Biomedical Imaging Resource, Mayo Foundation). Regions of interest were selected from cross-sectional images from the aorta and bilateral renal cortex and medulla.11 Average density of each sampled region was plotted as a function of time,11 and time-density curves were fitted with modified gamma-variate functions.13 The area and first moment of each vascular curve were obtained, and perfusion (in milliliters per minute per cubic centimeter of tissue) was calculated as previously detailed11–13: Area Under Tissue Curve × Area Under Aortic Curve × First Moment of Tissue Curve × 60.

Cortical and medullary volumes (in cubic centimeters) were calculated from the images by use of a program implemented in Analyze.12 RBF was subsequently calculated for each kidney as the sum of the products of its cortical and medullary perfusions and corresponding volumes.12

Biochemical Determinations

PRA was determined in both systemic and renal venous blood using a standard radioimmunoassay technique.6 PGF$_{2\alpha}$-isoprostanes and thiobarbituric acid–reactive substances (TBARS), plasma markers of a pro-oxidant redox status, were measured with enzyme immunoassay and spectrophotometric measurement of TBARS at 532 nm, respectively, as previously described.6,14 Tissue activities of the oxygen-radical scavengers glutathione peroxidase, catalase, copper-zinc form of superoxide dismutase (CuZn-SOD), and Mn-SOD were determined in homogenized flash-frozen renal cortical tissue. Briefly, glutathione peroxidase activity was assayed spectrophotometrically from the rate of oxidation of NADPH at 22°C, catalase activity from the reduction of hydrogen peroxide (rate of decrease of

### Table 1. Systemic Characteristics of Pigs Before and After Induction of Unilateral Renal Artery Stenosis vs Normal, Sham-Operated Pigs

<table>
<thead>
<tr>
<th>Group Characteristics</th>
<th>Baseline</th>
<th>5 Weeks</th>
<th>10 Weeks</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, kg</td>
<td>24.5±1.9</td>
<td>34.3±1.6*</td>
<td>53.5±4.0*</td>
<td>59.5±3.0*</td>
</tr>
<tr>
<td>Degree of stenosis, %</td>
<td>...</td>
<td>62.6±10.7</td>
<td>70.0±0.3</td>
<td>...</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>113±5</td>
<td>141±11†</td>
<td>144±10‡</td>
<td>103±6</td>
</tr>
<tr>
<td>PRA, ng·ml$^{-1}$·h$^{-1}$</td>
<td>0.4±0.1</td>
<td>3.4±1.9</td>
<td>0.4±0.1</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$-isoprostanes, pg/mL</td>
<td>125±12</td>
<td>157±21‡</td>
<td>176±23‡</td>
<td>106±9</td>
</tr>
<tr>
<td>TBARS, nmol/mL</td>
<td>...</td>
<td>...</td>
<td>3.9±0.5†</td>
<td>2.1±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM, n=7 per group at baseline and 10 weeks; n=6 per group at 5 weeks. Normal values were obtained 10 to 12 weeks after baseline.

*P=0.05 vs baseline; †P=0.05 vs normal in corresponding study.
absorbance at 240 nm) at 25°C, and SOD activity on the basis of the spontaneous autoxidation of pyrogallol at 25°C (with formation of end products at an absorbance peak at 420 nm) as previously described. To distinguish between the CuZn-SOD and Mn-SOD isoenzymes, parallel measurements were performed in the presence of 1 μmol/L KCN, a selective inhibitor of CuZn-SOD. All tissue activities were normalized for protein content by the Lowry method.

Statistical Analysis
Quantitative values are expressed as mean±SEM. Statistical comparisons between different experimental periods or groups were performed using ANOVA and Student’s t test and regressions by the least-squares fit. Statistical significance was accepted at P=0.05.

Results
Systemic Measurements
At baseline, levels of MAP, PRA, and RBF in group 1 (Figure 1; Tables 1 and 2) were similar to levels in group 2 (99.4±6.5 mm Hg, 0.6±0.3 ng · mL⁻¹ · h⁻¹, and 396±67 mL/min; P=NS). By 5 weeks after the procedure, significant renal artery stenosis in group 1 (Table 1) was associated with a decrease in stenotic RBF (Table 2) compared with both single-kidney RBF of group 2 at their 5-week study (535±37 mL/min; P=0.008) and contralateral kidney (Table 2; P=0.01). MAP increased by 29% (Figure 1; P=0.005). PRA strongly tended to increase (Figure 1; P=0.06), lateralized to the stenotic kidney, and directly correlated with MAP (r=0.63; P=0.037). By 10 weeks of RVH, stenotic kidney RBF was still decreased, contralateral RBF was significantly higher than in group 2 (Table 2), and MAP remained elevated (Table 1). However, PRA returned to baseline levels (Figure 1; P=0.3 versus baseline) and no longer correlated with MAP (r=−0.14; P=0.77).

Baseline systemic levels of isoprostanes were similar in both groups. For the 5-week measurement, only 6 (rather than 7) samples of isoprostanes were available for analysis in group 1. At that time, PGF₂α-isoprostanes (n=6) significantly increased in this group (Figure 1; P=0.04) and correlated with both the increase in MAP (Figure 2a, left; r=0.82; P=0.047) and PRA (Figure 2a, right; r=0.83; P=0.04). By 10 weeks, systemic isoprostanes (n=7) tended to increase further (Figure 1; P=0.07 compared with 5 weeks), and still strongly tended to correlate with the increased MAP (Figure 2b, left; r=0.75; P=0.07), but not PRA (Figure 2b, right; r=−0.36; P=0.4). Systemic TBARS levels were also significantly elevated at that time compared with normal (Table 1; P=0.005). Renal vein isoprostanes of both kidneys showed similar trends as their systemic levels (Table 2), which were stronger for total compared with free levels of PGF₂α-isoprostanes (data not shown), and did not lateralize at any experimental period.

Group 2 showed no significant changes in MAP, PRA, or PGF₂α-isoprostanes among the experimental periods, although their RBF increased significantly at 5 and 10 weeks compared with basal levels, probably because of growth.

Renal Parenchymal Measurements
Both the stenotic and contralateral kidneys in group 1 showed a significant and similar decrease postmortem in renal cortical levels of the oxygen-radical scavengers glutathione peroxidase, catalase, CuZn-SOD, and Mn-SOD versus group 2 (Table 2; P<0.01 for all the measurements).

Discussion
The present study demonstrates that experimental RVH due to unilateral renal artery stenosis is accompanied by a progressive increase in systemic plasma levels of PGF₂α-isoprostanes that continues to parallel the increase in MAP even after PRA returns to baseline levels. This was associated with decreased endogenous radical-scavenger levels in both stenotic and contralateral kidneys. The present study indicates a role for increased systemic oxidative stress in the pathogenesis of RVH and for enhanced renal oxidation-sensitive mechanisms in the pathogenesis of ischemic and hypertensive renal injury.

We recently demonstrated that experimental porcine renal artery stenosis could be achieved by percutaneous deployment of an intra-arterial balloon-expandable coil that leads to
progressive luminal narrowing, simulating the development of human renovascular lesions. Renal tissue damage in this model can be observed only in the most severe stenoses, probably because of the relatively short duration of intervention and the absence of comorbid conditions. We also previously showed that plasma Ang II levels in this model correlated well with both MAP and PRA, whereas during the chronic phase, they no longer correlated with PRA.

TABLE 2. Single-Kidney Characteristics in Pigs Before and After Induction of Unilateral Renal Artery Stenosis (n=7) vs Normal, Sham-Operated Pigs

<table>
<thead>
<tr>
<th>Group Characteristics</th>
<th>Baseline</th>
<th>5 Weeks</th>
<th>10 Weeks</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal blood flow, mL/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenotic kidney</td>
<td>340±38</td>
<td>296±73†</td>
<td>444±95†</td>
<td>661±41*</td>
</tr>
<tr>
<td>Contralateral kidney</td>
<td>312±23</td>
<td>637±67*</td>
<td>814±101†</td>
<td></td>
</tr>
<tr>
<td>Renal vein PRA, ng·mL⁻¹·h⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenotic kidney</td>
<td>0.7±0.1</td>
<td>10.1±7.9</td>
<td>1.0±0.6</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>Contralateral kidney</td>
<td>0.5±0.1</td>
<td>2.3±1.1</td>
<td>0.4±0.0</td>
<td></td>
</tr>
<tr>
<td>Renal vein PGF₂α-isoprostanes, pg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenotic kidney</td>
<td>103±17</td>
<td>143±17</td>
<td>146±17</td>
<td>108±9.3</td>
</tr>
<tr>
<td>Contralateral kidney</td>
<td>98.5±18</td>
<td>138±23</td>
<td>154±15*</td>
<td></td>
</tr>
<tr>
<td>Renal tissue (mU/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenotic</td>
<td>66.1±1.7†</td>
<td>58.0±1.6†</td>
<td>87.8±2.3</td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>16.9±1.0†</td>
<td>13.0±0.6†</td>
<td>22.4±1.5</td>
<td></td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>6.7±0.1†</td>
<td>7.3±0.1†</td>
<td>8.1±0.1</td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>2.6±0.1†</td>
<td>2.1±0.1†</td>
<td>3.4±0.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM, n=7 per group at baseline and 10 weeks; n=6 per group at 5 weeks. *P<0.05 vs baseline, †P<0.05 vs normal in respective study.
present study, by 1 month after induction of RVH, this model exhibited an increase in PRA that was typical of an early phase of RVH and lateralized to the stenotic renal vein. The fact that the increase in systemic PRA was not statistically significant (Table 1) but showed a strong trend probably reflects the variability in time course and severity of response among animals. PRA in the contralateral renal vein was also elevated, possibly because of high circulating renin levels, because the unclipped kidneys in Goldblatt hypertension showed suppressed intrarenal renin mRNA but Ang II–dependent renal function.19 This was paralleled by an increase in both systemic and bilateral renal-vein levels of PGE2-isoprostanes. After 10 weeks of observation, the degree of stenosis showed little progress and MAP remained elevated. However, PRA returned to the normal levels typical for a chronic phase of RVH. Nonetheless, the increase in isoprostanes was maintained and still paralleled the sustained increase in MAP. RBF of the contralateral kidney was also decreased in both studies, underscoring the hemodynamic consequences in the stenotic kidney. After 10 weeks of observation, the degree of stenosis showed little progress and MAP remained elevated. However, PRA returned to the normal levels typical for a chronic phase of RVH. Nonetheless, the increase in isoprostanes was maintained and still paralleled the sustained increase in MAP. RBF of the contralateral kidney was also decreased in both studies, underscoring the hemodynamic consequences in the stenotic kidney.

The significant correlation between PRA and PGE2-isoprostanes observed during the early phase of RVH in the present study supports a link between the renin-angiotensin system and oxidative-mediated pathways. Ang II is a known stimulus for generation of reactive oxygen species, which may reduce the bioavailability of nitric oxide and produce isoprostanes through oxidation of arachidonic acid.8 However, in contrast to its early phase, during the chronic stage of RVH, PRA normalized and no longer correlated with either isoprostanes or MAP. Remarkably, plasma levels of PGE2-isoprostanes remained elevated and continued to parallel the increase in MAP. In this setting, an increase in isoprostanes possibly could be achieved when bioavailability of nitric oxide is concurrently decreased or MAP simultaneously elevated, thereby constituting a powerful mechanism that potentially could augment vascular sensitivity to Ang II. This is underscored by our recent suggestion that enhanced vasoconstriction to low levels of Ang II could also result from a cascade of events related to increased oxidative stress.2 Furthermore, activation of the tissue (rather than systemic) renin-angiotensin system may also contribute to a pro-oxidant shift in this setting. Nevertheless, hypertension per se might induce release of reactive oxygen species that could modulate a vicious cycle of several interdependent partici-
pants, and the present study cannot exclude the possibility that PGE2-isoprostane formation resulted from increased MAP rather than Ang II.

Because both increased Ang II generation and ischemia promote formation of oxygen radicals, oxidation-sensitive mechanisms could have been activated mainly in the stenotic kidney. However, as opposed to renal-vein PRA, PGE2-isoprostanes levels did not lateralize to the side of the stenotic kidney at any experimental period and were in fact more increased in the contralateral renal vein at 10 weeks of RVH. Hence, activation of these pathophysiological mechanisms could have taken place in both kidneys, in the systemic vasculature, or elsewhere. Furthermore, tissue measurements also argued against the stenotic kidney as the sole locus of increased oxidative stress, because both kidneys showed a decrease of endogenous radical scavengers, as can be observed in various forms of renal injury.26,27

Interestingly, the latter findings suggest a potential role for activation of redox-sensitive mechanisms not only in sustaining RVH, but also in evolving ischemic and hypertensive renal injury. Increased generation of reactive oxygen species is involved in the pathogenesis of various forms of renal dysfunction and damage14,28 and is one of the proposed mechanisms of Ang II–induced tissue damage.29 Ang II–driven superoxide generation promotes mesangial hypertrophy and extracellular matrix production and may cause membrane lipid oxidation and disruption of the structural integrity and capacity for cell transport and energy production.30 Activation of growth factors and cytokines and the mitogen-activated protein kinase/extracellular-regulated kinase cascade,34 may also play a role in the mechanism of intrarenal action of Ang II and reactive oxygen species.

In summary, we observed that in the early phase of RVH, an increase in PRA and MAP was associated with increased systemic oxidative, which continued to parallel and could have potentially mediated sustenance of MAP when systemic PRA later declined. Further studies will be needed to determine whether these factors are causally related to sustaining RVH. Oxidation-sensitive mechanisms were also activated in the tissue and may conceivably play a role in progression of ischemic and hypertensive renal injury observed in renal artery stenosis.

Acknowledgments

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