Blood Pressure, Blood Flow, and Oxygenation in the Clipped Kidney of Chronic 2-Kidney, 1-Clip Rats
Effects of Tempol and Angiotensin Blockade

Fredrik Palm, Maristela Onozato, William J. Welch, Christopher S. Wilcox

Abstract—Angiotensin II maintains renal cortical blood flow and renal oxygenation in the clipped kidney of early 2-kidney, 1-clip Goldblatt hypertensive (2K,1C) rats. The involvement of Ang II is believed to decline, whereas oxidative stress increases during the progression of 2K,1C hypertension. We investigated the hypothesis that the acute administration of drugs to inhibit reactive oxygen species (Tempol), angiotensin type 1 receptors (candesartan), or angiotensin-converting enzyme (enalaprilat) lowers mean arterial pressure and increases kidney blood flow and oxygenation in the clipped kidney of chronic 2K,1C rats in contrast to sham controls. Twelve months after left renal artery clipping or sham, mean arterial pressure, renal cortical blood flow, and renal cortical and medullary oxygen tension were measured after acute administration of Tempol followed by enalaprilat or candesartan followed by enalaprilat. The mean arterial pressure of the 2K,1C rat was reduced by candesartan (−9%) and, more effectively, by Tempol (−35%). All of the applied treatments had similar blood pressure–lowering effects in sham rats (average: −21%). Only Tempol increased cortical blood flow (+35%) and cortical and medullary oxygen tensions (+17% and +94%, respectively) in clipped kidneys of 2K,1C rats. Administration of enalaprilat had no additional effect, except for a modest reduction in cortical blood flow in the clipped kidney of 2K,1C rats when coadministered with candesartan (−10%). In conclusion, acute administration of Tempol is more effective than candesartan in reducing the mean arterial blood pressure and improving renal blood perfusion and oxygenation in the clipped kidney of chronic 2K,1C rats. (Hypertension. 2010;55:298-304.)

Key Words: Goldblatt hypertension  ■ renal oxygen tension  ■ renal blood flow  ■ Tempol  ■ angiotensin receptor blockers  ■ angiotensin-converting enzyme inhibitors

A reduced renal perfusion pressure after the clipping of a renal artery increases angiotensin II (Ang II) concentrations in both kidneys. There is an early development of Ang II–dependent hypertension. Ang II acting on Ang II type 1 receptors (AT₁-Rs) results in activation of superoxide production by the NADPH oxidase, which is abundantly expressed in the kidney. The involvement of oxidative stress in early 2-kidney, 1-clip Goldblatt hypertensive (2K,1C) rats has been demonstrated by prolonged administration of Tempol to reduce reactive oxygen species (ROS), which reduced the mean arterial pressure (MAP) and improved the renal blood flow and glomerular filtration rate and PO₂ of the clipped kidney. In contrast, the administration of an AT₁-R blocker (ARB) indeed reduced the MAP both during the early and the chronic phase of the clipped kidney. Therefore, therapeutic options for correcting hypertension and renal ischemia in the chronic phase of 2K,1C renovascular hypertension are presently limited. Nevertheless, prolonged Ang II infusion reduces renal tissue PO₂, which is ascribed to excessive formation of ROS. Several of the conditions commonly associated with increased oxidative stress display reduced kidney PO₂, including diabetes mellitus, lipopolysaccharide-induced sepsis, and ischemia-reperfusion injury. Therefore, we investigated the role of ROS in renal vasoconstriction and oxygenation in chronic 2K,1C hypertension.

Anderson et al proposed that increased Ang II in the early 2K,1C model is a homeostatic modification to provide sufficient glomerular capillary pressure to sustain the glomerular filtration rate (GFR). Indeed, we have reported that oxygen availability in clipped kidneys of early (3-week–old) 2K,1C rats is maintained by Ang II acting on Ang II type 2 receptors (AT₂-Rs), resulting in NO release. This may be of importance because chronic renal hypoxia and repeated episodes of renal ischemia may contribute to hypertension and progressive kidney disease. However, the long-term consequences of increased levels of Ang II and subsequent oxidative stress for the function of the clipped kidney of the 2K,1C model of renovascular hypertension are not currently
well understood. The role of Ang II evolves during 2K,1C hypertension. Therefore, the present work was designed to investigate the role of ROS and/or Ang II acting on AT1-Rs in maintaining CBF even in the chronic model. Therefore, temozolomide after candesartan (Figure 2), this suggested some role for AT2-Rs in maintaining CBF even in the chronic model. Therefore, we undertook a limited study of 2K,1C rats (n = 7) given the AT1-R antagonist PD-123,319 (1 mg · kg−1 · bolus + 1 mg · kg−1 · h−1; Sigma Aldrich; fully effective dose in acute 2K,1C rats) followed 30 minutes later by enalaprilat (0.3 mg · kg−1 · bolus + 0.3 mg · kg−1 · h−1) to test this hypothesis more directly.

### Materials and Methods

These studies were performed under guidelines recommended by the National Institutes of Health and approved by the Georgetown University Animal Care and Use Committee. As described in detail previously, young male Sprague-Dawley rats (80 to 100 g) were anesthetized with isoflurane (0.5% to 1.5%). A silver clip (0.2 mm) was placed around the left renal artery (2K,1C). Age-matched rats were used as controls (sham). All of the rats received hydralazine + hydrochlorothiazide + reserpine (HHR; 30.0 ± 10.0 ± 0.2 mg · kg−1 · day−1) in the drinking water, as described previously, to maximize survival of the clipped rats. The HHR treatment was discontinued 14 days before the acute experiments. Twelve months after clipping, all of the rats were anesthetized with Inactin (100 mg · kg−1 · IP; Sigma-Aldrich), and an endotracheal tube was inserted for spontaneous respiration. Rats were prepared and followed a similar protocol as those with acute 2K,1C hypertension, described in detail previously. Briefly, the left femoral artery was catheterized for monitoring MAP and the left femoral vein for infusion of saline (5 mL · kg−1 · h−1). The left kidney was immobilized in a plastic cup, whereas renal cortical PO2 and cortical blood flow (CBF) were measured with oxygen microelectrodes (Unisense) and laser-Doppler needle probes (Transonic Systems Inc), as described previously. The location of each measurement was visually confirmed at the end of the experiments by dissecting the kidneys under a microscope. Measurements were made before and after the administrations of candesartan (1 mg · kg−1 · bolus + 1 mg · kg−1 · h−1; kind gift from Astra Zeneca; manufacturer recommended dose for maximal inhibition of AT1-Rs in vivo; sham n = 7 and 2K,1C n = 8) followed after 30 minutes by enalaprilat (0.3 mg · kg−1 · bolus + 0.3 mg · kg−1 · h−1; Novaplus 1.25 mg · mL−1; Baxter Healthcare Corporation; fully effective antihypertensive dose in acute 2K,1C rats), or Tempol (174 μmol · kg−1 · bolus + 174 μmol · kg−1 · h−1; Sigma Aldrich; fully effective antihypertensive dose in spontaneously hypertensive rats; sham n = 7 and 2K,1C n = 7) followed after 30 minutes by enalaprilat (0.3 mg · kg−1 · bolus + 1 mg · kg−1 · h−1).

We selected this protocol, using candesartan followed by enalaprilat, for comparison with a previous series in acute 2K,1C rats where an increase in blood pressure or renal vascular resistance with enalaprilat in rats pretreated with candesartan was an indication of an AT1-R–mediated change. Because enalaprilat reduced CBF modestly after candesartan (Figure 2), this suggested some role for AT2-Rs in maintaining CBF even in the chronic model. Therefore, we undertook a limited study of 2K,1C rats (n = 7) given the AT2-R antagonist PD-123,319 (1 mg · kg−1 · bolus + 1 mg · kg−1 · h−1; Sigma Aldrich; fully effective dose in acute 2K,1C rats) followed 30 minutes later by enalaprilat (0.3 mg · kg−1 · bolus + 0.3 mg · kg−1 · h−1) to test this hypothesis more directly.

### Statistics

ANOVA was used to compare multiple data sets. When appropriate, this was followed by the Dunnett post hoc test. Two data sets within the same group were compared using Student t test for paired comparisons. Relative changes displayed in the figure are for visualization purpose only; statistics were calculated using the original parametric data sets (GraphPad Prism, GraphPad Software). For all of the comparisons, P < 0.05 was considered statistically significant. All of the values are expressed as mean ± SEM.

### Results

Fifty-four rats were clipped, and 24 rats survived until the acute experiments 12 months later, resulting in a survival ratio of 44%. Of the 24 remaining rats, 1 was excluded because of surgical errors and 1 because of having an infarcted and atrophied left kidney. All of the animals that underwent sham surgery survived.

The body weights did not differ between sham and 2K,1C rats (Table 1). Both the nonclipped and the clipped kidneys of 2K,1C rats were significantly heavier when corrected for body weight than kidneys of sham rats.

2K,1C rats had elevated MAP, averaging 163 ± 3 (n = 22) compared with sham 123 ± 3 (n = 14, P < 0.05; Figure 1 and Table 2). All of the acute interventions reduced the MAP modestly, but similarly, in elderly sham rats. The elevated MAP of 2K,1C rats was reduced by candesartan. However, Tempol was significantly more effective (Figure 1). Enalaprilat did not produce a further fall in MAP in any rats administered Tempol or candesartan.

The CBF was unchanged after all of the applied acute interventions in sham rats. Candesartan did not change CBF significantly in 2K,1C rats (Figure 2). However, CBF was increased in 2K,1C rats by Tempol. CBF did not further change in 2K,1C rats given enalaprilat after Tempol but was reduced significantly by enalaprilat after candesartan administration (Figure 2).

The baseline renal cortical PO2 was similar in 2K,1C and sham rats (Table 1). Candesartan, or the combination of candesartan and enalaprilat, increased cortical PO2 in sham rats, whereas Tempol had no effect in this group (Figure 3). Only Tempol increased the cortical PO2 in 2K,1C rats, after which there was no further change with enalaprilat (Figure 3).
The baseline renal medullary PO2 was reduced in 2K,1C rats compared with sham (Table 1). Renal medullary PO2 was increased after all of the applied acute interventions in sham, whereas again only Tempol increased the medullary PO2 in 2K,1C rats (Figure 4). Subsequent administration of enalapril after Tempol did not alter the medullary PO2 further.

The 2K,1C rats administered PD-123 319 had similar body weight (49±21 g), kidney:body weight ratios (right kidney: 3.71±0.82; P<0.05), right:left kidney weight ratio (0.76), and baseline cortical and medullary PO2 (44±1 and 16±1 mm Hg, respectively) as the other two 2K,1C groups. Administered PD-123 319 to 2K,1C rats had no effect on any of the investigated parameters. Subsequent addition of enalaprilat caused a modest reduction of MAP (Table 2).

**Discussion**

The main new findings from this study are that the acute administration of Tempol is more effective in lowering MAP in chronic 2K,1C rats than blockade of the renin-angiotensin system with ARB or angiotensin-converting enzyme (ACE) inhibitor. Furthermore, only Tempol increased the blood flow and the tissue PO2 in the clipped kidney, whereas the ARB had no effect. The addition of an ACE inhibitor to rats that had received Tempol or candesartan did not improve these responses.

Both the hypertension and the intrarenal alterations in blood flow and tissue PO2 in early 2K,1C hypertension are highly dependent on increased Ang II action. Although we found that the acute inhibition of AT1-Rs or ACE reduced the blood pressure in chronic 2K,1C rats, the fall in blood pressure was significantly greater after a reduction in ROS with Tempol. Furthermore, only Tempol effectively increased both the CBF and the tissue PO2 in the renal cortex and medulla in the clipped kidney of chronic 2K,1C rats, whereas interventions directed against the renin-angiotensin system had no effect on these parameters. The altered function of the clipped kidney likely is a response to prolonged exposure to severely increased Ang II levels and to a reduced perfusion pressure and medullary PO2. Chronically elevated Ang II can induce self-sustaining mechanisms that maintain ROS production. Thus, prolonged Ang II can reduce the antioxidant defense systems, such as superoxide dismutase; upregulate NADPH oxidase subunits; oxidize tetrahydrobiopterin with subsequent uncoupling of NO synthases; stabilize the thromboxane-prostanoid receptors; and induce vascular and renal inflammation, to name but a few. The results from the present study show that the effects of Tempol differ substantially from those that follow interruption of the renin-angiotensin system, which is consistent with the concept of a self-sustaining ROS-producing system.

Tempol, whether given by acute intravenous infusion22 or by a 2-week subcutaneous infusion7 into rats with early 2K,1C renovascular hypertension, reduced both the MAP and the renal vascular resistance substantially. Oral administration of Tempol for 223 or 524 weeks to rats with early 1K,1C renovascular hypertension also reduced the MAP. However, although we had found a reduced renal cortical PO2 in the clipped kidney of early 2K,1C hypertensive rats, the cortical PO2 was maintained in the clipped kidney in the chronic model. The tissue oxygen availability is determined by the interplay between oxygen delivery and con-

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**Table 2. Response in MAP, Relative CBF and Kidney PO2 to Administration of the Selective AT2-R Blocker PD-123 319 and the Combination of PD-123 319 + Enalaprilat in a Separate Group of 2K,1C Rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After PD-123 319</th>
<th>After PD-123 319 + Enalaprilat</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>162±5</td>
<td>155±5</td>
<td>133±5*</td>
</tr>
<tr>
<td>CBF, % of baseline</td>
<td>100</td>
<td>101±1</td>
<td>88±9</td>
</tr>
<tr>
<td>Cortical PO2, % of baseline</td>
<td>100</td>
<td>105±3.3</td>
<td>104±5</td>
</tr>
<tr>
<td>Medullary PO2, % of baseline</td>
<td>100</td>
<td>96±11</td>
<td>91±17</td>
</tr>
</tbody>
</table>

All values are mean±SEM of n=7.

*P<0.05 vs baseline.
consumption. The latter is highly influenced by the tubular Na\textsuperscript{+}/H\textsuperscript{+} transport\textsuperscript{25} and, therefore, by the GFR. Although we did not measure the GFR, previous studies have established that there is a reduced GFR in the clipped kidney of chronic 2K,1C hypertensive rats that has been ascribed to a reduced renal perfusion pressure.\textsuperscript{26} However, the absence of an atrophic stenotic kidney in this study implies that the increase in systemic pressure in these rats likely overcame the obstruction caused by the clip and that the blood perfusion distal to the mild or moderate obstruction must have been fairly well maintained. If so, it is also likely that the GFR of the clipped kidney would have been substantially higher compared with previous reports.\textsuperscript{26} A well-maintained blood supply may also explain the near-normal PO\textsubscript{2} values in the kidney cortex of the clipped kidney in the present model.

In contrast to the kidney cortex, we detected a significantly reduced PO\textsubscript{2} in the outer medulla of the clipped kidneys of chronic 2K,1C hypertensive rats. This might contribute to progressive kidney dysfunction, as proposed in other models of hypoxia-induced kidney damage.\textsuperscript{15,18} Therefore, it is important to elucidate the mechanisms involved and to identify potential interventions to restore the PO\textsubscript{2} in the medulla of the clipped kidney. The results from the present study show that Tempol not only reduced the MAP but effectively increased the blood flow and cortical and medullary PO\textsubscript{2} in the clipped kidney. These effects were not influenced by subsequent administration of an ACE inhibitor, which implies that Tempol had corrected any effects attributed to ongoing Ang II generation, but the mechanism was not studied further in these experiments. It could entail a reduction by Tempol of

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**Figure 2.** Mean changes in renal CBF in sham and 2K,1C rats during the different acute interventions. *P<0.05 when compared with baseline with the same group. †P<0.05 when compared with the candesartan or the combined candesartan + enalaprilat treatment within the same category of animals. All of the values are mean±SEM.

**Figure 3.** Mean changes in renal cortical PO\textsubscript{2} in sham and 2K,1C rats during the different acute interventions. *P<0.05 when compared with baseline with the same group. †P<0.05 when compared with the candesartan or the combined candesartan + enalaprilat treatment within the same category of animals. All of the values are mean±SEM.
renal medullary superoxide with enhanced NO bioavailability, which would increase blood flow and thereby oxygen delivery and also increase the efficiency of mitochondria to produce ATP. NO reversibly inhibits mitochondrial respiration by competing for the binding site of oxygen. This effect of NO is potentiated at the low PO2 levels recorded in the medulla.

Chade et al reported that an acute intrarenal infusion of Tempol into pigs fed a high-fat diet and studied 12 weeks after a renal artery stenosis failed to improve the reduced levels of renal blood flow, cortical perfusion, or GFR. In contrast, we detected large increases in renal blood flow and cortical perfusion in rats studied at an even more prolonged stage of 2K,1C renovascular disease. It is possible that the 10-fold increase in low-density lipoprotein cholesterol in the pigs fed a high-fat diet, which was accompanied by extensive perivascular and tubulointestinal fibrosis, and neovascularization, which was not pronounced in the kidneys in our study, had limited the hemodynamic response to Tempol in the hypercholesterolemic pig model. Alternatively, the rather modest degree of renal artery stenosis, which did not induce renal atrophy in our rat model, may have provided an opportunity for a reduction in ROS to be apparent as an increase in blood flow and oxygenation.

Ang II induces NO release via AT2-Rs within the clipped kidney in early 2K,1C rats and thereby sustains blood flow and PO2 in that kidney. Indeed, enalaprilat did reduce CBF modestly in the clipped kidney after candesartan, consistent with a residual role for AT2-Rs in maintaining cortical flow, if not oxygenation in the chronic model. However, the selective AT2-R blocker PD-123 319 had no effect on MAP, CBF, or kidney oxygenations, whereas the effects of enalaprilat persisted after AT2-Rs blockade. Therefore, we conclude that the involvement of AT2-Rs is not very evident in the kidneys of rats with chronic 2K,1C. The reason for this change in the importance of the AT2-Rs is presently unknown. In contrast to the AT2-Rs, Tempol retains its efficiency from the early to the chronic 2K,1C phase of modest hypertension in the rat.

The present study has some limitations, mainly relating to methodologic difficulties. Because of the massive fibrosis resulting from the clip placed on the renal artery, it is very difficult to measure total kidney blood flow and split-kidney function in these rats. We, therefore, choose to use laser-Doppler methodology, because the main focus of this study was to measure hemodynamic alterations occurring in response to acute drug administrations. The laser-Doppler technique is suitable for acute experiments, as described previously, if care is taken to confirm each measurement location. However, the disadvantage is that the hemodynamic significance of the clip cannot be verified, as discussed further above. A second limitation was the apparently rather modest degree of renal artery stenosis engendered, which in fact was accompanied by renal hypertrophy rather than renal atrophy. Furthermore, all of the animals were chronically treated with HHR throughout the course of the study to maximize survival of the clipped animals. It is also possible that only animals with only a modest renal artery restriction survived until the actuate experiments 12 months later and that this influenced the results of the present study. Although previous studies have shown that HHR indeed lowers blood pressure, this treatment has been shown to have only a marginal influence on the development of renal alterations, such as albuminuria, glomerulosclerosis, cytokine levels, renal blood flow response to blockade of the NO system, kidney oxygen tension, and intrarenal levels of Ang II and angiotensinogen in several different models of hypertension-induced kidney damage, including rats with spontaneous hypertension, 5/6 nephrectomized, deoxycorticosterone-acetate hypertension, and 2K,1C rats. These previous reports, together with the fact that the HHR therapy was discontinued 14 days before the acute experiments, indicate that this procedure should have no major influence on the results reported in the present study.

Figure 4. Mean changes in renal medullary PO2 in sham and 2K,1C rats during the different acute interventions. *P<0.05 when compared with baseline with the same group. †P<0.05 when compared with the candesartan or the combined candesartan+enalaprilat treatment within the same category of animals. All of the values are mean±SEM.
In conclusion, acute administration of Tempol had superior antihypertensive efficiency and improvement of renal blood perfusion and oxygenation compared with an ARB in this model. Whether this will translate into differences in long-term renoprotection during ischemic nephropathy warrants further study.

Perspectives

Unilateral renal artery stenosis in humans can lead to hypertension, renal atrophy, and reduced renal function. At present, no treatments have been found to prevent progressive kidney dysfunction in this setting. A controlled trial of renal artery angioplasty in patients with renal artery stenosis found no beneficial effects on GFR at 1 year. Current pharmacological therapy is equally unsatisfactory. ACE inhibitors and ARBs are effective in reducing blood pressure but can worsen renal insufficiency. Chronic renal hypoxia has been considered an underlying cause of progressive chronic kidney disease. Thus, our finding that the acute administration of Tempol is effective both in reducing the blood pressure and in improving the renal oxygenation in the clipped kidney at both the early and the chronic phases suggests a possible role for drugs of this class in the prevention or management of hypertension and renal insufficiency, at least in patients with modest degrees of renal artery stenosis. This warrants further study.

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References


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