Exacerbation of Chronic Renovascular Hypertension and Acute Renal Failure in Heme Oxygenase-1–Deficient Mice

Philippe Wiesel,* Anand P. Patel,* Ivirth M. Carvajal, Zhi Yuan Wang, Andrea Pellacani, Koji Maemura, Nicole DiFonzo, Helmut G. Rennke, Matthew D. Layne, Shaw-Fang Yet, Mu-En Lee,† Mark A. Perrella

Abstract—Heme oxygenase (HO) is a cytoprotective enzyme that degrades heme (a potent oxidant) to generate carbon monoxide (a vasodilatory gas that has anti-inflammatory properties), bilirubin (an antioxidant derived from biliverdin), and iron (sequestered by ferritin). Because of properties of HO and its products, we hypothesized that HO would be important for the regulation of blood pressure and ischemic injury. We studied chronic renovascular hypertension in mice deficient in the inducible isofor of HO (HO-1) using a one kidney–one clip (1K1C) model of disease. Systolic blood pressure was not different between wild-type (HO-1+/+), heterozygous (HO-1+/−), and homozygous null (HO-1−/−) mice at baseline. After 1K1C surgery, HO-1+/+ mice developed hypertension (140±2 mm Hg) and cardiac hypertrophy (cardiac weight index of 5.0±0.2 mg/g) compared with sham-operated HO-1+/+ mice (108±5 mm Hg and 4.1±0.1 mg/g, respectively). However, 1K1C produced more severe hypertension (164±2 mm Hg) and cardiac hypertrophy (6.9±0.6 mg/g) in HO-1−/− mice. HO-1−/− mice also experienced a high rate of death (56%) within 72 hours after 1K1C surgery compared with HO-1+/+ (25%) and HO-1+/− (28%) mice. Assessment of renal function showed a significantly higher plasma creatinine in HO-1+/− mice compared with HO-1−/− mice. Histological analysis of kidneys from 1K1C HO-1−/− mice revealed extensive ischemic injury at the corticomedullary junction, whereas kidneys from sham HO-1+/− and 1K1C HO-1+/− mice appeared normal. Taken together, these data suggest that chronic deficiency of HO-1 does not alter basal blood pressure; however, in the 1K1C model an absence of HO-1 leads to more severe renovascular hypertension and cardiac hypertrophy. Moreover, renal artery clipping leads to an acute increase in ischemic damage and death in the absence of HO-1. (Circ Res. 2001;88:1088-1094.)

Key Words: hypertension ■ ischemia ■ oxidative injury ■ endothelin

Heme oxygenase (HO) is the enzyme that catalyzes the initial reaction in heme catabolism.1 The inducible isofrom, HO-1, is upregulated by diverse stimuli including mediators of oxidative stress.2,3 HO-1 is a cytoprotective enzyme4–6 that degrades heme (a potent oxidant) to generate carbon monoxide (CO, a vasodilatory gas that has anti-inflammatory properties), bilirubin (an antioxidant derived from biliverdin), and iron (sequestered by ferritin). Because of properties of HO-1 and its products, it is believed that HO-1 may play an important role in cellular antioxidant defense mechanisms.

Sacerdoti et al7 and Escalante et al8 have demonstrated that either acute or chronic administration of an inducer of HO-1 (stannous chloride) to spontaneously hypertensive rats led to a normalization of blood pressure. Other inducers of HO-1 or HO substrates have also been shown to decrease blood pressure in hypertensive rats.9–11 This response is not limited to the systemic vasculature, because inducers of HO-1 can prevent the development of hypoxic pulmonary hypertension.12 In addition, it has been demonstrated that treatment of normal13 or endotoxemic14 rats with inhibitors of HO (metalloporphyrins) produces an increase in systemic arterial pressure. Because biliverdin itself has not been associated with the regulation of blood pressure,13 these studies provided evidence that CO via the HO system may contribute to the regulation of systemic blood pressure. One way in which CO regulates blood pressure is by producing cGMP,2,15–17 which has vasodilatory properties.

Beyond the vasodilatory effect of CO through cGMP, Morita and Kourembanas18 have shown that vascular...
smooth muscle cell–derived CO inhibits production of the potent vasoconstrictor endothelin (ET)-1. This inhibition may contribute to the effects of CO on vascular tone and blood pressure. Investigators have also demonstrated that angiotensin II–induced hypertension promotes an induction of HO-1,19–21 suggesting that upregulation of endogenous HO-1 may attempt to counteract the hypertensive effect of angiotensin II.

An organ that plays a predominant role in the chronic regulation of blood pressure is the kidney. Interestingly, several lines of evidence suggest that beyond its potential effects on systemic vascular tone and blood pressure, HO-1 modulates renal function.22 HO-1 is induced in rat models of acute renal injury including glycerol-induced renal failure,5 nephrotoxic serum nephritis,23 cisplatin nephropathy,24 and ischemia/reperfusion–induced renal failure.25,26 Increased expression of HO-1 has been noted in renal tubules,23 renal glomeruli,27 and inflammatory cells infiltrating the kidney,28 depending on the model studied. Moreover, in some of these models, chemical inhibitors of HO activity have been shown to worsen renal damage,5,24,26 suggesting a protective role for HO-1. Unfortunately, these inhibitors are not selective for HO-1, they affect HO-2 and other enzyme systems, and they may have undesirable side effects.29 Thus, the generation of HO-1 null mice30,31 allows a means to specifically investigate the role of HO-1 in different disease processes.

To evaluate the role of HO-1 in the control of systemic blood pressure and renal protection, we used a one kidney–one clip (1KIC) model of renovascular hypertension.32,33 This model consists of a unilateral nephrectomy and a partial occlusion of the renal artery of the remaining kidney that leads to a reduction in renal perfusion. In the 1KIC model, fluid retention by the single stenotic kidney leads to volumedependent hypertension. The more recent development of this model in mice33 allows for the study of renovascular hypertension in HO-1 null mice.

### Materials and Methods

#### Mouse Model of Renovascular Hypertension

Mice that were wild-type (+/+), heterozygous (+/-), or homozygous null (-/-) for targeted disruption of HO-131 were studied. These mice were maintained on a 129Sv/BALB/c genetic background, and littermates were used for the studies. One kidney-one clip surgery was performed on mice that were 5 weeks of age, as previously described.33 Briefly, a 0.12-mm clip was inserted on the left renal artery to chronically reduce perfusion pressure, and a right nephrectomy was performed. As controls, mice also underwent the same procedure, with the exception that no clip was applied. The mice were killed 28 hours or 9 weeks after surgery. Kidney tissue for mRNA analysis and plasma samples for creatinine analysis were collected and stored at −80°C and −20°C, respectively, until further processing. Because ET-1 may play a detrimental role in ischemia-induced renal injury,34–36 pilot studies were performed to select the most optimal dose of ET-A receptor antagonist to study, using doses of 2.5 and 5.0 mg/kg. From these pilot studies, the ET-A receptor antagonist BQ-123 (Calbiochem-Novabiochem Corp) was administered intraperitoneally (5.0 mg/kg IP) 2 hours before and 12 hours after 1KIC surgery. The Harvard Medical Area Standing Committee on Animals approved the protocol.

#### Blood Pressure Measurements and Assessment of Cardiac Weight Index

A tail-cuff method was used to measure systolic blood pressure (SBP), as described previously.37 Mice were trained by placing them in restraints, 1 hour daily, for 10 days before the experiments. Once fully trained, conscious mice were restrained and gently warmed using a heating lamp. An occlusion cuff and a piezoelectric pulse sensor were placed around their tail (Kent Scientific), and SBP was measured after a 15-minute acclimatization period. A minimum of eight serial measurements was made and the average value was calculated (Mac Lab software, version 3.5, AD Instruments). Both training and blood pressure measurements were performed at the same time each day (afternoon). Nine weeks after surgery, the mice were killed and their hearts removed. Cardiac weight index (CWI) was calculated as CWI=heart weight (mg)/body weight (g).

#### Biochemical Measurement of Creatinine

Twenty-eight hours after surgery, blood was obtained from the retro-orbital sinus, centrifuged at 2500g for 10 minutes at 4°C, and stored at −20°C. Plasma creatinine (Cr) levels were measured using a commercial kit (Sigma), according to the manufacturer’s recommendations.

#### Northern Blot Analysis

Total RNA was obtained from mouse kidneys by guanidinium isothiocyanate extraction and silica-gel-membrane spin technology (RNeasy midi kit, Qiagen). RNA was fractionated on a 1.3% formaldehyde-agarose gel and transferred to nitrocellulose filters. The filters were hybridized at 68°C for 2 hours with a 32P-labeled rat HO-1 probe or a mouse ET-1 probe in QuikHyb solution (Stratagene) as described previously.37 The hybridized filters were then washed in 30 mmol/L sodium chloride, 3 mmol/L sodium citrate, and 0.1% SDS at 55°C and autoradiographed with Kodak XAR film. To assess for differences in RNA loading, the filters were washed in a 50% formamide solution at 80°C and rehybridized with a 32P-labeled oligonucleotide probe complementary to 18S ribosomal RNA.

#### Histological and Immunohistochemical Analysis

Kidneys were harvested from the mice 28 hours after 1KIC surgery, washed in PBS, and fixed in 10% formalin overnight at 4°C. The specimens were processed, embedded, and sectioned at a thickness of 5 μm. Immunocytochemical staining was performed next. To reduce nonspecific binding, the sections were incubated in cadence buffer (Shandon) containing 10% normal goat serum. Rabbit polyclonal antibody against rat HO-1 (SPA895, StressGen Biotechnologies) was applied for 1 hour at room temperature and then overnight at 4°C at a dilution of 1:400. Sections were rinsed twice with cadence buffer and incubated with biotinylated goat anti-rabbit IgG at a dilution of 1:200 for 1 hour at room temperature. They were then rinsed with cadence buffer twice and incubated with avidin-biotin complex ( Vectastain ABC kit, Vector Labs) for 1 hour at room temperature. After washing twice with cadenza buffer, the tissue sections were developed using the Vector DAB substrate kit (Vector Laboratory) and counterstained with 1% methyl green. The presence of HO-1 was indicated by the development of a brown color. Periodic acid-Schiff (PAS) staining was also performed on the kidney sections, as previously described.38

#### Statistics

Where indicated, comparisons between groups were made by factorial ANOVA followed by Fisher’s least-significant difference test when appropriate. Survival comparisons between groups were made by the χ² goodness-of-fit test. Statistical significance was accepted at P<0.05. Data are expressed as mean±SEM.

### Results

#### Enhanced Renovascular Hypertension and Cardiac Hypertrophic Response in HO-1−/− Mice

Under basal conditions, SBP was not different between HO-1+/+ (106±3 mm Hg), HO-1−/− (99±2 mm Hg), and HO-1−/− (101±2 mm Hg) mice. Mice were then challenged with the 1KIC model of renovascular hypertension. We
measured SBP 9 weeks after surgery in sham and 1K1C mice (Figure 1). In sham-operated mice, SBP was similar in HO-1+/+, HO-1−/−, and HO-1+/− mice (108±5, 105±4, 107±4 mm Hg, respectively). As expected, 1K1C surgery led to a chronic increase in SBP in HO-1+/+ mice (140±2 mm Hg) and a similar increase in HO-1−/− mice (130±2 mm Hg). However, SBP was significantly higher in 1K1C HO-1−/− mice (164±2 mm Hg). These data suggest that although not necessary for the maintenance of normotension in intact mice, HO-1 may play a compensatory role in chronic renovascular hypertension.

CWI was also measured to assess cardiac hypertrophy in the three genotypes of mice after sham or 1K1C surgery. In all three genotypes, 1K1C mice developed increased CWI compared with their sham controls (Figure 2). However, similar to the SBP response, HO-1−/− mice developed more severe cardiac hypertrophy (6.9±0.6 mg/g) than HO-1+/+ (5.0±0.2 mg/g) and HO-1+/− (5.3±0.1 mg/g) mice after 9 weeks of 1K1C-induced renovascular hypertension (Figure 2). Whereas CWI was increased in HO-1−/− mice, total body weight was not different (P=0.64, n=6 to 9 mice/genotype) between HO-1+/− mice (22.3±1.0 g), HO-1+/+ (22.7±1.2 g), and HO-1+/− (23.8±0.8 g) mice.

**Figure 1.** Effect of HO-1 on the development of chronic renovascular hypertension. SBP was measured using a tail-cuff method 9 weeks after 1K1C (black bars) or sham (white bars) surgery in HO-1+/+, HO-1−/−, and HO-1+/− mice. n=4 to 5 mice/genotype in each surgical group. *P<0.05 vs sham controls; †P<0.05 vs all other groups.

**Figure 2.** Effect of HO-1 on the cardiac hypertrophy response to chronic renovascular hypertension. Nine weeks after 1K1C (black bars) or sham (white bars) surgery, HO-1+/+, HO-1−/−, and HO-1+/− mice were killed and their hearts removed. CWI was calculated as CWI=heart wet weight (mg)/body weight (g). n=4 to 5 mice/genotype in each surgical group. *P<0.05 vs sham controls; †P<0.05 vs all other groups.

### Mortality Rate in HO-1+/+, HO-1+/−, and HO-1−/− Mice After 1K1C or Sham Surgery

<table>
<thead>
<tr>
<th>HO-1 Genotype</th>
<th>Surgery</th>
<th>Acute Mortality Rate (&lt;72 Hours, %)</th>
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<tr>
<td>HO-1+/+</td>
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<tr>
<td></td>
<td>1K1C</td>
<td>25</td>
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<td>1K1C</td>
<td>28</td>
</tr>
<tr>
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<td>Sham</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1K1C</td>
<td>56*</td>
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*P<0.05 vs 1K1C HO-1+/+ and HO-1+/− mice.

### Increased Mortality Rate in HO-1−/− Mice After 1K1C Surgery

In the original report on the 1K1C model of renovascular hypertension in mice, an acute mortality rate of ~25% was observed after surgery. These animals developed renal infarctions and subsequent organ failure. Strikingly, acute mortality rate was markedly higher in HO-1−/− mice (56%) compared with HO-1+/+ (25%) and HO-1+/− (28%) mice after 1K1C surgery (Table). Within 72 hours after the 1K1C procedure, 14 of 25 mice in the HO-1−/− group died. Acute mortality rate was not increased in sham-operated 1K1C HO-1−/− mice. In fact, there were no deaths in sham-operated mice of any group. This increased mortality rate was not restricted to the early time points after 1K1C surgery, because the mortality rate increased to 84% in HO-1−/− mice after 9 weeks, whereas no late deaths (after 72 hours) were noted in the HO-1+/+ and HO-1+/− mice.

### Acute Renal Failure in HO-1−/− Mice After 1K1C Surgery

The finding that a reduction in renal perfusion leads to an increased mortality rate in HO-1−/− mice prompted us to evaluate renal function shortly after clipping. We chose to assess the mice 28 hours after surgery because we have witnessed anephric mice die as early as 28 hours postoperatively. Moreover, we focused on HO-1−/− mice (not different from HO-1+/− mice) compared with HO-1+/− mice. In sham-operated HO-1−/− and HO-1+/− mice, plasma Cr concentration was not different between the two groups (32.9±1.3 versus 32.7±0.7 μmol/L respectively, Figure 3). After 1K1C surgery, plasma Cr concentration did not increase significantly in HO-1−/− mice (46.7±6.2 μmol/L); however, it increased markedly in HO-1−/− mice (83.3±17.2 μmol/L). These data suggest that kidneys from HO-1−/− mice are more susceptible to ischemic injury.

### Regulation of HO-1 and ET-1 Gene Expression After 1K1C Surgery

To further investigate the role of HO-1 in the adaptation of the kidney to a reduction in perfusion, we assessed the renal expression of HO-1 mRNA 28 hours after clipping HO-1−/− mice. Whereas HO-1 was expressed only at a low level in sham-operated mice, it was significantly induced after 1K1C surgery in HO-1−/− mice (Figure 4A).

Studies have demonstrated that ET-1 may play a detrimental role in the course of acute renal failure. Because
HO-1-derived CO is known to inhibit the expression of ET-1,18 we hypothesized that in HO-1−/− mice there may be an induction of ET-1 after 1K1C surgery. We performed Northern blot analysis to evaluate renal ET-1 mRNA levels 28 hours after 1K1C or sham surgery. ET-1 mRNA was expressed at low levels in HO-1+/− and HO-1−/− mice after sham surgery (Figure 4B), and renal artery clipping did not induce ET-1 mRNA at this time point in HO-1−/− mice. In contrast, ET-1 mRNA was induced in 1K1C HO-1−/− mice (Figure 4B).

**Effect of ET A Receptor Antagonist on Acute Renal Failure in HO-1−/− Mice After 1K1C Surgery**

Administration of an antagonist to the ET A receptor (ET A RA) had no effect on plasma Cr concentrations of HO-1+/− mice after renal artery clipping (Figure 5). However, the increase in plasma Cr in HO-1−/− mice (95.4±18.6 μmol/L) after 1K1C surgery was prevented by administration of the ET A RA (49.4±14.4 μmol/L). Moreover, all HO-1−/− mice receiving ET A RA (n=9) survived the acute period after renal artery clipping.

**HO-1 Expression and Kidney Damage Associated With 1K1C Surgery**

Kidneys were harvested from the mice 28 hours after 1K1C surgery. Immunohistochemical staining was then performed for HO-1 (Figures 6A and 6B). After 1K1C surgery, increased expression of HO-1 was noted in the renal tubules of HO-1−/− mice (Figure 6B, brown staining, arrows). Interestingly, staining was not seen in the glomeruli.

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**Figure 3.** Renal response to 1K1C surgery in the presence or absence of HO-1. Twenty-eight hours after surgery, mice were killed and plasma was collected. Plasma Cr concentrations were subsequently measured in HO-1+/− (black bars) and HO-1−/− (white bars) mice after 1K1C (+) or sham (−) surgery, n=4 in each of the sham HO-1+/− and HO-1−/− groups and n=8 in each of the 1K1C HO-1+/− and HO-1−/− groups. *P<0.05 vs all other groups.

**Figure 4.** Regulation of HO-1 and ET-1 mRNA levels after 1K1C surgery. Twenty-eight hours after 1K1C or sham surgery, total RNA was extracted from kidneys. Northern blot analyses were performed and the nitrocellulose filters were hybridized with 32P-labeled rat HO-1 (A, HO-1+/− mice) or mouse ET-1 (B, genotypes as labeled) probes. Each lane on the Northern blots (A and B) represents kidney tissue from an individual mouse. Filters were also hybridized with a 32P-labeled oligonucleotide probe complementary to 18S ribosomal RNA to assess for differences in loading.

**Figure 5.** Effect of an ET A RA on 1K1C-induced acute renal failure in the absence of HO-1. Twenty-eight hours after 1K1C surgery, mice were killed and plasma was collected. Plasma Cr concentrations were subsequently measured in HO-1+/− mice in the absence (−, black bar) or presence (+, gray bar) of ET A RA. Plasma Cr levels were also assessed in HO-1−/− mice in the absence (−, white bar) or presence (+, striped bar) of ET A RA. n=4 in each of the HO-1+/− and HO-1−/− groups not receiving ET A RA and n=9 in each of the HO-1+/− and HO-1−/− groups receiving ET A RA. *P<0.05 vs HO-1+/− mice not receiving ET A RA.

**Figure 6.** Histological assessment of HO-1 expression and kidney damage associated with 1K1C surgery. Kidneys were harvested from the mice 28 hours after 1K1C surgery. Immunohistochemical staining was then performed (A and B) using an antibody against rat HO-1. Brown staining demonstrates HO-1 immunostaining (arrows). PAS staining was also performed (C through F) on kidney tissue in the presence (+) or absence (−) of an antagonist to ET A RA in HO-1+/− and HO-1−/− mice. Arrowheads denote the corticomedullary junction of the kidney in panels C through F.
PAS staining was next performed (Figures 6C through 6F) on kidney tissue from HO-1\(^{-/-}\) and HO-1\(/^{+/-}\) mice in the presence (+) or absence (−) of an antagonist to ET\(_{A}\)RA. In HO-1\(/^{+/-}\) mice in which the expression of HO-1 is increased by renal artery clipping, 1K1C surgery did not induce ischemic damage in the presence or absence of the ET\(_{A}\)RA (Figures 6C and 6E). However, in HO-1\(/^{−/−}\) mice, clipping of the renal artery produced ischemic damage predominating in the renal tubules of the outer medulla (Figure 6D). The architecture of the corticomedullary junction (Figure 6D, arrowheads) was distorted in HO-1\(/^{−/−}\) mice with evidence of acute tubular necrosis in comparison with HO-1\(/^{+/-}\) mice (Figures 6C, arrowheads). Administration of ET\(_{A}\)RA, 5.0 mg/kg IP, before and 12 hours after 1K1C surgery, prevented this ischemic damage (Figure 6F).

**Discussion**

HO-1 has been implicated in the control of blood pressure and the regulation of vascular tone. Thus, one goal of this study was to determine the importance of endogenous HO-1 on blood pressure regulation. No difference in SBP was evident between HO-1\(/^{+/-}\), HO-1\(/^{−/−}\), and HO-1\(/^{−/−}\) mice at baseline (see Results and Figure 1, sham mice). These data revealed that different from the acute inhibition of HO enzymes in normal animals,\(^1\) the chronic absence of HO-1 does not lead to a sustained increase in SBP. This may suggest a role for HO-2 in blood pressure regulation in the setting of acute HO-1 inhibition, or that during the chronic absence of HO-1 compensatory mechanisms prevent an increase in SBP. We next studied the effect of HO-1 absence on a model of renovascular hypertension. In the 1K1C model, one kidney is removed while the remaining kidney undergoes arterial constriction. In response to this decrease in renal perfusion pressure, plasma renin levels rapidly rise with a subsequent increase in circulating angiotensin II levels.\(^3\) This results in the early hypertensive response. Chronically, the 1K1C procedure leads to volume retention by the single clipped kidney and a volume-dependent, low-renin hypertensive phenotype.\(^3\) Because HO-1 expression is regulated by the administration of angiotensin II\(^1\) and that inducers of HO-1 normalize blood pressure in models of hypertension,\(^7\) we hypothesized that endogenous HO-1 may attempt to counteract the development of renovascular hypertension. Indeed, in the absence of endogenous HO-1, there was an exacerbation of chronic renovascular hypertension after the 1K1C procedure (Figure 1).

Associated with the hypertension of renal artery clipping, the myocardium undergoes a hypertrophic response as an adaptation to increased blood pressure.\(^3\) As shown in Figure 2, cardiac mass was significantly greater in HO-1\(/^{−/−}\) mice compared with HO-1\(/^{+/-}\) and HO-1\(/^{−/−}\) mice. This response paralleled the hypertensive response shown in Figure 1. However, Seki et al\(^1\) have shown in spontaneously hypertensive rats that chronic administration of an inducer of HO-1 (stannous chloride), at a dose that does not alter systemic blood pressure, can attenuate the development of cardiac hypertrophy. This may suggest that beyond a regulation of systemic blood pressure, HO-1 may have independent effects on the development of cardiac hypertrophy. Important to the development of renovascular hypertension, Raju et al\(^4\) have previously shown that bilateral renal artery ischemia followed by reperfusion of the kidneys can induce HO-1 expression and increase cGMP levels in the heart. It was speculated that hemodynamic stress caused by occlusion of the renal arteries led to activation of HO-1 gene expression in the heart.

Depending on the severity of ischemia, renal artery occlusion can lead to injury and dysfunction of the kidney. Ischemic renal injury is characterized by intrarenal vasoconstriction, leading to reduced glomerular plasma flow and filtration rate, and reduced oxygen delivery to the tubules of the outer medulla.\(^4\) HO has been implicated as a mediator of medullary blood flow.\(^5\) However, this renal ischemic response is often attributed to the release of endogenous vasoconstrictors, such as ET-1.\(^6\) The importance of ET-1 in ischemia-induced acute renal failure has been demonstrated by the beneficial effects of ET receptor antagonists on the pathophysiological consequences of this process.\(^3\) In our study, renal artery clipping led to an induction of HO-1 mRNA (Figure 4A), and increased HO-1 protein was localized to the renal tubules of HO-1\(/^{−/−}\) mice (Figure 6B). In the setting of this HO-1 induction, ischemia induced by the renal artery clipping was not severe enough to cause an acute increase in plasma Cr levels (Figure 3) or structural damage to the kidney (Figure 6C). However, in the absence of HO-1, mice subjected to the same clipping experienced an increased mortality rate (Table), increased plasma Cr levels (Figure 3), and ischemic damage to the renal tubules of the outer medulla (Figure 6D). By administering an antagonist to ET\(_{A}\)RA, the increase in plasma Cr (Figure 5) and the ischemic damage (Figure 6F) were prevented. Taken together, these data suggest that in the absence of HO-1 and the presence of increased renal ET-1, kidneys are at increased risk for acute ischemic damage and subsequent failure leading to death. Because the 1K1C model of renovascular hypertension is a volume-dependent process initiated by a limitation in renal function, we believe that the exacerbated hypertension in HO-1\(/^{−/−}\) mice reflects progressive renal injury contributed to by elevated levels of renal ET-1. More severe tubular injury and renal failure have also been demonstrated in HO-1\(/^{−/−}\) mice subjected to the glycerol model of heme protein toxicity\(^4\) and cisplatin-induced nephrotoxicity.\(^5\)

In the present study, the predispisition for ischemic renal failure in HO-1\(/^{−/−}\) mice may well relate to an environment prone to vasoconstriction in the absence of HO-1–derived CO and the increase in renal ET-1 levels. Moreover, because HO-1 plays an important role in cellular antioxidant defense mechanisms, the absence of HO-1 leads to increased susceptibility to tissue oxidative damage.\(^3\) HO-1 is an important mediator of inflammation\(^2\) that may contribute to renal injury in the setting of ischemia,\(^6\) and HO-1–derived CO has recently been suggested to have anti-inflammatory effects through a pathway involving mitogen-activated protein kinases.\(^9\)

In summary, data from our study suggest that chronic deficiency of HO-1 does not alter basal blood pressure; however, in the 1K1C model an absence of HO-1 leads to more severe renovascular hypertension and cardiac hypertrophy. Moreover, renal artery clipping leads to increased...
ischemic damage and death in the absence of HO-1, and ET-1 appears to play an important role in the pathophysiology of this acute renal ischemic damage. These data provide further support for the importance of endogenous HO-1, a cytoprotective enzyme, in the regulation of cardiovascular function and the mediation of pathophysiological stimuli leading to oxidative stress.

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References

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