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

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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 isoform 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 isoform, 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 (transnous 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, 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. HO-1 is induced in rat models of acute renal injury including glycerol-induced renal failure, nephrotoxic serum nephritis, cisplatin nephropathy, and ischemia/reperfusion–induced renal failure. Increased expression of HO-1 has been noted in renal tubules, renal glomeruli, and inflammatory cells infiltrating the kidney, depending on the model studied. Moreover, in some of these models, chemical inhibitors of HO activity have been shown to worsen renal damage, 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. Thus, the generation of HO-1 null mice 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 (1K1C) model of renovascular hypertension. 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 1K1C model, fluid retention by the single stenotic kidney leads to volume-dependent hypertension. The more recent development of this model in mice 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-1 were studied. These mice were maintained on a 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. 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 Northern Blot Analysis was obtained from the mice 28 hours after 1K1C 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. Immunohistochemical staining was performed next. To reduce nonspecific binding, the sections were incubated in cadenza 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 1:200. Sections were rinsed twice with wash buffer and incubated with biotinylated goat anti-rabbit IgG at a dilution of 1:400. Sections were rinsed twice with wash buffer and incubated with peroxidase–conjugated streptavidin-biotin complex (Vectastain ABC kit, Vector Labs) for 1 hour at room temperature. After washing twice with wash 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.

**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 1K1C 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.

In the original report on the 1K1C model of renovascular hypertension in mice,33 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.

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

In the original report on the 1K1C model of renovascular hypertension in mice,33 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.34 Because
HO-1-derived CO is known to inhibit the expression of ET-1,18 so 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<sub>A</sub> Receptor Antagonist on Acute Renal Failure in HO-1+/− Mice After 1K1C Surgery

Administration of an antagonist to the ET<sub>A</sub> receptor (ET<sub>A</sub>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<sub>A</sub>RA (49.4 ± 14.4 μmol/L). Moreover, all HO-1−/− mice receiving ET<sub>A</sub>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.
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, 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 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. 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 response. Because HO-1 expression is regulated by the adaption of angiotensin II and that inducers of HO-1 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 and cisplatin-induced nephrotoxicity.

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 development of renovascular hypertension, Raju et al have previously shown that bilateral renal artery ischemia followed by reperfusion of the kidneys can induce HO-1 expression and increase GMP 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. HO has been implicated as a mediator of medullary blood flow. However, this renal ischemic response is often attributed to the release of endogenous vasoconstrictors, such as ET-1. 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. 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 and cisplatin-induced nephrotoxicity.
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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