Heme oxygenase, a novel target for the treatment of hypertension and obesity?

Peter A. Hosick and David E. Stec

Department of Physiology and Biophysics, Center for Excellence in Cardiovascular-Renal Research, University of Mississippi Medical Center, Jackson, Mississippi

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Hosick PA, Stec DE. Heme oxygenase, a novel target for the treatment of hypertension and obesity? Am J Physiol Regul Integr Comp Physiol 302: R207–R214, 2012. First published November 9, 2011; doi:10.1152/ajpregu.00517.2011.—Heme oxygenase (HO) is the rate-limiting enzyme in the metabolism of heme-releasing bioactive molecules carbon monoxide (CO), biliverdin, and iron, each with beneficial cardiovascular actions. Biliverdin is rapidly reduced to bilirubin, a potent antioxidant, by the enzyme biliverdin reductase, and iron is rapidly sequestered by ferritin in the cell. Several studies have demonstrated that HO-1 induction can attenuate the development of hypertension as well as lower blood pressure in established hypertension in both genetic and experimental models. HO-1 induction can also reduce target organ injury and can be beneficial in cardiovascular diseases, such as heart attack and stroke. Recent studies have also identified a beneficial role for HO-1 in the regulation of body weight and metabolism in diabetes and obesity. Chronic HO-1 induction lowers body weight and corrects hyperglycemia and hyperinsulinemia. Chronic HO-1 induction also modifies the phenotype of adipocytes in obesity from one of large, cytokine producing to smaller, adiponectin producing. Finally, chronic induction of HO-1 increases oxygen consumption, CO2, and heat production and activity in obese mice. This review will discuss the current understanding of the actions of the HO system to lower blood pressure and body weight and how HO or its metabolites may be ideal candidates for the development of drugs that can both reduce blood pressure and lower body weight.

Heme oxygenase (HO) is an important enzyme responsible for the catabolism of heme into ferrous iron, carbon monoxide (CO), and biliverdin. Biliverdin is then converted to bilirubin by the ubiquitous enzyme biliverdin reductase. The iron released is rapidly sequestered by ferritin to limit oxidative damage. HO enzymes are present in all cells in the body in two major forms, the constitutively expressed isoform HO-2 and the inducible isoform HO-1. The HO-1 isoform can be induced by a wide range of physiological stimuli, such as hypoxia and shear stress; but more importantly, it is induced in numerous pathological conditions, such as exposure to toxins, heavy metals, inflammation, and other forms of tissue injury (1). The metabolites of the HO reaction, CO and bilirubin, also have important functions in the cardiovascular system. CO is an important gaseous transmitter and a vasodilator of blood vessels. CO also has important antiapoptotic and anti-inflammatory actions as well. Bilirubin is a potent antioxidant that can directly scavenge oxidants, such as superoxide anion as well as inhibit the production of superoxide anion through direct inhibition of NAD(P)H oxidase. Mild elevations in plasma bilirubin levels have been demonstrated to protect against numerous cardiovascular diseases, such as heart attack and stroke in large population studies.

Obesity is a major risk factor for the development of diabetes and cardiovascular disease, such as hypertension and stroke. Obesity is also associated with an increased risk of injury from acute trauma as well as an increased incidence of the development of cancer (6, 9, 55, 66). HO-1 induction both systemically as well as centrally has been demonstrated to result in chronic decreases in body weight. HO-1 induction also has a positive effect on metabolic disturbances in obesity, such as hyperglycemia and hyperinsulinemia. Recent studies have explored the effect of HO-1 induction on the physiology of adipocytes, including the differentiation of mesenchymal stem cells (MSC) into adipocytes as well as the profile of hormones released from adipocytes. The results of these studies indicate the induction of HO-1 and increases in its associated metabolites, CO and bilirubin, may offer novel therapeutic targets for the treatment of both hypertension and obesity. In addition, induction of HO-1 could be most beneficial to treat the increasing cases of metabolic syndrome, an emerging disease of obesity, diabetes, dyslipidemia, and hypertension.

HO and Hypertension

Anti-hypertensive effects of HO-1 induction. The effect of HO-1 induction on the development of hypertension has a long history. Early studies using a rat model of genetic hypertension, the spontaneously hypertensive rat, demonstrated that induction of HO-1 with tin prevented the development of hypertension in this model (59). This initial finding was repli-
Another potential mechanism by which increases in HO-1 can lower blood pressure is through decreases in superoxide production in the renal medulla (Fig. 1). Studies in cultured mouse thick ascending loop of Henle (TALH) cells have demonstrated that increases in HO-1 protein as well as in CO or bilirubin alone can attenuate ANG II-dependent increases in superoxide production (30). Superoxide has been demonstrated to increase sodium reabsorption in the TALH through direct mechanism as well as through decreases in nitric oxide (NO) (Fig. 1) (23). Interestingly, decreases in bilirubin production via targeting of biliverdin reductase with siRNA in TALH cells increases ANG II-dependent superoxide production, as well as sodium reabsorption (80). Superoxide derived from the TALH can also decrease the levels of NO, which can have effects on blood flow through tubulovascular crosstalk (42). These studies suggest that antioxidant functions may play an important role in the blood pressure-lowering actions of HO-1 induction in the kidney.

**HO metabolites and hypertension.** Evidence for the antihypertensive actions of HO-1 induction has been presented above; however, HO metabolites CO and bilirubin can also have beneficial actions to lower blood pressure on their own. It has previously been shown that low-level CO inhalation of 60 ppm for 2 h/day for 2 wk resulted in a significant attenuation in the development of ANG II-dependent hypertension in mice (32). Decreases in blood pressure in mice receiving CO were also associated with lowering of ANG II-stimulated vascular superoxide production. This would suggest that CO may lower blood pressure in part by counteracting the effects of ANG II to increase superoxide production in both renal tubules and the vasculature. While the results of this study are very promising, further studies into the mechanism by which chronic low level CO exposure can lower blood pressure are needed. For example, how important are increases in cellular cGMP levels to this antihypertensive action of low-dose CO inhalation? cGMP can act in tubules to decrease sodium reabsorption as well as in the
vasculature to promote vasodilatation (Fig. 2A) (28, 43). There are several other pathways that can also be influenced by CO, such as MAPK, signal transducers and activators of transcription, and phosphatidylinositol 3-kinase-Akt, all of which could mediate the blood pressure-lowering response to chronic increases in CO. Chronic increases in CO could also be achieved by administration of CO-releasing molecules (CORMs). CORMs have been previously demonstrated to be effective in protecting the kidney against ischemia-induced renal injury and renal injury due to administration of nephrotoxins (67, 73). Whether CORMs are effective in lowering blood pressure in established models of hypertension is not currently known and is an area that merits further investigation.

The results from several large human population studies have indicated a correlation between moderate increases in plasma bilirubin levels (50% to 2-fold) and protection from cardiovascular disease (7, 8, 26, 27). Despite the large amount of correlative data, the mechanism by which moderate increases in plasma bilirubin afford protection against cardiovascular disease is unknown. The hyperbilirubinemic Gunn rat, which lacks the necessary enzyme (UGT1A1) responsible for conjugation of bilirubin in the liver has been demonstrated to be protected against several forms of hypertension including ANG II-dependent and DOCA salt-induced hypertension (45, 53). However, the plasma levels of bilirubin in the Gunn rat are many fold higher (>20) than the levels exhibited by patients in the upper quartile of plasma bilirubin in the human population studies. Given the large increases in plasma bilirubin exhibited in Gunn rats, this model is not appropriate for determining the mechanism by which moderate 50% to twofold increases in plasma bilirubin can lower blood pressure and protect against the development of cardiovascular disease. Recently, a model of chronic, moderate hyperbilirubinemia was developed in the mouse by two methods: treatment with the UGT1A1 competitive inhibitor indinavir and treatment with UGT1A1 antisense DNA, which increased total plasma bilirubin levels 50% and levels of unconjugated bilirubin by two- to sixfold compared with control mice (76). Moderately hyperbilirubinemic mice are resistant to ANG II-induced hypertension and also exhibit less ANG II-mediated increases in vascular superoxide production, as well as increases in plasma nitrate levels reflecting increases in NO bioavailability (72). Moderate hyperbilirubinemia is also able to reverse ANG II-mediated decreases in renal blood flow and glomerular filtration rate (76). These results suggest that moderate hyperbilirubinemia may lower blood pressure through modifications in renal vascular function, possibly through decreases in vascular superoxide production and increases in NO in the renal vasculature (Fig. 2B). Bilirubin may also modify vascular reactivity through mechanisms independent of its antioxidant actions. Bilirubin can attenuate ANG II-mediated increases in preproendothelin mRNA in cultured mouse endothelial cells as well as effect endothelin-mediated calcium influx in immortalized vascular smooth muscle cells (D.E. Stec, unpublished observations) (Fig. 2B). The results of the moderate hyperbilirubinemia studies support hepatic UGT1A1 as a potential target for the development of antihypertensive therapies. It is also possible that other genes in the liver responsible for the conjugation of bilirubin could also be potential targets for the development of additional antihypertensive drugs that act via increasing plasma bilirubin levels. However, careful consideration of the appropriate target(s) is warranted to minimize any potential side effects due to disruption of drug metabolism or other important hepatic functions.

**HO and Obesity**

**HO and body weight.** The first evidence of the relationship between HO-1 induction and body weight was derived from studies in which CoPP, an HO-1 inducer, was administered to rats either subcutaneously or via intracerebroventricular injection (15, 18, 19, 21). Results from these studies demonstrated that administration of CoPP either by a single, high dose (10 or 25 μmol/kg body wt) or with weekly treatment with a lower dose (1 μmol/kg body wt) resulted in sustained body weight loss of between 20 and 25% compared with rats receiving vehicle (19, 21). Similar effects on body weight were also observed when CoPP was administered via intracerebroventricular injection (19, 20). CoPP administration either via subcutaneous or intracerebroventricular administration led to an initial decrease in food intake of between 60 and 80%, compared with vehicle-treated animals. While this initial decrease in food intake can explain the immediate weight loss in CoPP-treated animals, the effect of CoPP on food intake is not sustained chronically, with food intake returning to normal levels in ~30 days (19–21). These results indicate that CoPP...
is able to cause sustained weight loss independently of long-term changes in food intake, possibly through changing the set point for body weight via a central mechanism (22). Actions of systemic CoPP administration on body weight are dependent on HO-1 induction as evidenced by studies that have demonstrated coadministration of an HO inhibitor significantly attenuates weight loss in male ob/ob mice (34). Interestingly, HO-1 induction may exhibit a sex-dependent effect on weight loss in obesity as induction of HO-1 with CoPP failed to demonstrate significant decreases in body weight in female versus male leptin-deficient ob/ob mice (5). Induction of HO-1 via other compounds has also been demonstrated to induce weight loss. The apolipoprotein mimetic peptide L-4F, which is a strong inducer of HO-1, has been demonstrated to lower body weight in both ob/ob as well as leptin receptor-deficient db/db mice (51, 52, 71). Treatment with hemin, also an inducer of HO-1, has been demonstrated to lower body weight in leptin receptor-deficient, Zucker diabetic fatty rats (49). Finally, treatment with chromium histidinate, which induces HO-1 through the nuclear factor erythroid 2-related factor 2 (Nrf2)-dependent pathway was found to prevent weight gain in Wistar rats fed a high-fat diet (69).

While it is clear that induction of HO-1 both centrally and systemically can prevent the development of obesity, the mechanism by which HO-1 induction elicits weight loss is not known. It has been speculated that central induction of HO-1 with CoPP may change the set point for body weight regulation via changes in the central levels of signaling molecules and hormones such as NO and neuropeptide Y (17, 35). The precise areas of the brain that mediate the anorectic effect of chronic central HO-1 induction are not known, but previous studies have implicated the medial (paraventricular, dorsomedial, and ventromedial) nuclei of the hypothalamus as potential mediators of this response (22). The mechanism responsible for weight loss after systemic injection of CoPP is also not known. One mechanism that can be ruled out is the ability of systemically administered HO-1 inducers to increase HO-1 levels in the brain. As shown in Fig. 3A, chronic administration of CoPP at 5 mg/kg for 21 wk failed to induce HO-1 in brain homogenates from lean normal and obese melanocortin 4 receptor (MC4R)-deficient (loxTB) mice yet resulted in significant attenuation of body weight gain in two models of MC4R receptor deficiency (12). Interestingly, the levels of HO-1 in the brain are elevated in obese loxTB-deficient mice; a finding that is also true for other mouse models of genetic obesity, as well as for dietary-induced obesity in mice (Fig. 3B). It is not currently known whether increased levels of HO-1 in the brain are a compensatory response to weight gain in general. The functional significance of this finding needs to be further tested in models in which the central induction of HO-1 is prevented. This could be achieved by the development of tissue-specific transgenic or gene knockout HO-1 mice in which the levels of HO-1 could be altered in a specific population of cells or nuclei in the brain. Systemic HO-1 induction may result in decreases in body weight through several mechanisms described in detail below; however, the relative importance of HO-1 induction in organs, such as adipose tissue, liver, and muscle is not known and may require the development of novel animal models in which HO-1 levels can be specifically altered in each of these tissues.

HO and metabolism. Systemic induction of HO-1 has been reported to have many beneficial actions on metabolic disorders in obesity. Induction of HO-1 lowers hyperglycemia and hyperinsulinemia in several models of obesity in both rats and mice (34, 47, 49, 51, 52). Improvements in these parameters are associated with increased levels of phosphorylated AMP-activated protein-kinase (AMPK), Akt, and insulin receptors as well as increased abundance of the glucose transporter-4 in both adipocytes and skeletal muscle (Fig. 3) (12, 47). AMPK phosphorylation in adipose tissue results in increased fatty acid oxidation and glucose uptake and decreased lipogenesis, lypolysis, and triglyceride synthesis (13). Recently, it was reported that induction of HO-1 in obese female ob/ob mice failed to significantly lower body weight but resulted in normalization of hyperglycemia and hyperinsulinemia and lowering of blood pressure (5). These results indicate that even in the absence of any significant changes in body weight induction of HO-1 in peripheral tissues may be beneficial to reverse hyperglycemia and insulin resistance in obesity through alterations in the AMPK/Akt signaling pathways.

HO-1 induction with CoPP either centrally or systemically results in transient decreases in food intake; yet, weight loss is sustained despite return of food intake to normal levels (19–21, 51). One explanation for this phenomenon is the effects of HO-1 induction on basal metabolism. A recent study, in mouse models of genetic obesity due to MC4R deficiency, demonstrated that chronic HO-1 induction with systemic low-dose CoPP administration results in increased oxygen consumption, CO₂, and heat production (12). These results indicate that increases in basal metabolism may underlie the sustained weight loss in response to chronic HO-1 induction. One potential target of HO-1 induction, which may be responsible for the increase in metabolism, is the mitochondria. HO-1 has been demonstrated to localize to the mitochondria where it can
affect the levels of mitochondrial transport proteins (10, 14, 63). The HO metabolite CO is an important regulator of mitochondrial biogenesis acting through the nuclear coactivator peroxisome proliferator-activated receptor-γ (PPARγ) coactivator-1α (PGC-1α) and Nrf2 pathways (Fig. 4) (54, 64). Through these pathways, CO can increase mitochondrial biogenesis, resulting in increased mitochondrial mass in peripheral tissues that could in turn increase in O2 consumption (56). However, this hypothesis needs to be specifically tested in obese animals in which HO-1 is chronically induced. It is also not known whether central HO-1 induction has similar effects on either O2 consumption or CO2 and heat production in obesity.

HO and the adipocyte. One of the most impressive aspects of chronic HO-1 induction in obesity is its effect on adipose tissue. Chronic HO-1 induction has been demonstrated to both limit the amount of adiposity and to significantly alter the phenotype of adipocytes (5, 12, 34, 51). Both visceral and subcutaneous fat from obese mice is mainly composed of a small number of large, cytokine-producing adipocytes; however, after chronic induction of HO-1, adipocytes appear smaller and more numerous (Fig. 5) (5, 52). Adipocytes from obese rodents are associated with increased production of inflammatory cytokines such TNF-α, IL-6, and IL-1β, all of which are decreased following chronic HO-1 induction (Fig. 5). Chronic HO-1 induction is also associated with increased plasma and adipose levels of adiponectin (Fig. 5) (5, 12, 48, 51). Recent studies have indicated that adiponectin deficiency is linked to increased inflammation and insulin resistance (16, 62). Specific studies in adiponectin-deficient mice have demonstrated an important role for adiponectin in the development of renal disease in obesity (61). Therefore, increased levels of adiponectin may be a mechanism by which induction of HO-1 results in improvement in insulin resistance and decreased inflammation in models of obesity.

Obesity is becoming recognized as a condition associated with increased levels of inflammation (3, 38, 40, 41). Inflammation has been linked to the development of insulin resistance and may contribute to target organ injury in obesity. Systemic HO-1 induction reduces levels of inflammatory cytokines, such as TNF-α, IL-6, and IL-1β levels, in both the plasma and adipose tissue of obese rats and mice (5, 34, 49, 51). Systemic induction of HO-1 also decreases the levels of NF-κB, which is an important transcription factor for genes involved in inflammation and the production of oxidants. HO-1 induction may also lower inflammatory cytokine levels through increases in either phosphorylated AMPK or PPARγ, both of which have anti-inflammatory properties. HO-1 can also limit inflammation through decreases in monocyte chemoattractant protein-1, which is involved with recruitment of inflammatory cells (44, 58). It is not known whether central induction of HO-1 results in similar decreases in markers of inflammation in obesity.

Another area in which HO induction could be beneficial in obesity is by preventing the creation of adipose tissue from MSC. Studies in human bone marrow MSC have demonstrated that decreases in HO with specific siRNAs increases adipocyte differentiation, an effect that was mimicked by high levels of glucose (70). Further studies have demonstrated that treatment of MSC with epoxyeicosatrienoic acids also decreases adipogenesis through increases in both HO and adiponectin (31). HO in MSC may also have beneficial actions apart from effects on adipogenesis as a recent study demonstrated the genetically modified MSC expressing HO-1 were able to increase capillary density and expression of angiogenic growth factors after acute myocardial infarction (82). Further studies have indicated that HO-1 induction in MSC may be an important therapeutic

**Fig. 4. HO-1 and metabolic signaling pathways.** HO-1 induction results in increased levels of phosphorylated AMPK, Akt, mammalian target of rapamycin (mTOR), insulin receptors, and increase levels of the glucose transporter GLUT-4 in skeletal muscle and adipose tissue. HO-1 induction also results in increased generation of CO which can act through PPARγ and nuclear respiratory factor (Nrf)-2 increasing mitochondrial biogenesis, which can result in increased oxygen consumption. HO-1 can also localize to the mitochondria where it can increase the levels of mitochondrial transport proteins resulting in increased free fatty acid transport (FFA trans.) to the inner mitochondrial membrane (IMM).

**Fig. 5. HO-1 and the adipocyte.** Adipocytes from obese individuals are usually few and large in size. These adipocytes usually produce large amount of cytokines, such as TNF-α, IL-6, and IL-1β. While these large adipocytes can produce inflammatory cytokines, they produce very little of the beneficial adipokine, adiponectin. HO-1 induction alters the physical appearance of adipocytes from few large adipocytes to many smaller adipocytes. These modified adipocytes exhibit increases in AMPK, peroxisome proliferator-activated receptor-γ (PPARγ), and adiponectin, while exhibiting decreased production of inflammatory markers and cytokines, such as monocyte chemoattractant protein-1 (MCP-1), NF-κB, TNF-α, and IL-6.
treatment option for myocardial ischemia, acute kidney injury, and pulmonary hypertension (37, 68, 81). These results suggest an important role for HO and its metabolites in the regulation of MSC differentiation and suggest that MSC genetically modified to overexpress HO-1 may have significant therapeutic potential to combat complications from obesity, such as heart and kidney disease.

**Perspectives**

The antihypertensive actions of HO induction have been known for many years now (59). It has only been recently that the mechanism by which induction of HO-1 lowers blood pressure has been examined. Effects of chronic HO-1 induction on body weight and metabolism have also been established, but the mechanisms linking induction of HO-1 to lowering of body weight have not been thoroughly explored. Recent advances have also demonstrated a potentially important role for HO-1 metabolites, CO, and bilirubin, in mediating the actions of HO-1 induction, as well as serving as potential therapeutic targets on their own. We are now ready to consider alternative approaches to increase HO-1 levels for therapeutic purposes, including 1) development of gene therapy-based strategies in which the levels of HO-1 protein could be increased; 2) targeting of transcriptional activators of HO-1, such as the Nrf2/Keap1 pathway to increase the levels of HO-1 protein (50, 65); and 3) targeting of transcriptional repressors of HO-1, such as Bach1 and micro-RNAs (miR217 and -377) to increase the levels of HO-1 protein (10). Similar approaches to further investigate the blood pressure lowering and metabolic actions of HO metabolites, CO and bilirubin, also need to be developed. A new generation CO donor that could be activated in vivo, as well as drugs that target bilirubin conjugation in the liver, offer novel therapeutic opportunities to combat the metabolic syndrome epidemic of which we are now in the midst.

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**DISCLOSURES**

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**AUTHOR CONTRIBUTIONS**

P.A.H. and D.E.S. prepared figures; P.A.H. and D.E.S. drafted manuscript; P.A.H. and D.E.S. edited and revised manuscript; P.A.H. and D.E.S. approved final version of manuscript.

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