Commentary

Heme Oxygenase-1 in Tissue Pathology

The Yin and Yang

Zheng Dong,* Yan Lavrovsky,[†]
Manjeri A. Venkatachalam,* and Arun K. Roy[†]

From the Departments of Pathology and Cellular & Structural Biology,[†] University of Texas Health Science Center at San Antonio, San Antonio, Texas*

Heme is a versatile molecule in nature and serves as the prosthetic moiety for numerous hemoproteins involved in oxygen delivery, electron transfer, and signal transduction.¹ However, when left unattended, the same heme can promote free radical formation and lipid peroxidation, resulting in cell damage and tissue injury.²⁻⁴ Thus, a fine balance between heme synthesis and catabolism is essential for the maintenance of cellular homeostasis. Whereas biosynthesis of heme is catalyzed by multiple enzymes, the only physiological mechanism of heme degradation is through heme oxygenase (HO).¹ Heme oxygenase catalyzes breakdown of the protoporphyrin ring, producing biliverdin, carbon monoxide, and free ferrous iron.⁵ To this date, three isoforms of heme oxygenase have been identified.⁶ Among them, HO-1, unlike the other two (HO-2 and -3), shows limited expression under normal situations and is induced by a variety of physiological stimuli.^{5,7,8} Heme, free iron, and a number of oxidative stressors can markedly potentiate this inductive response. Transcriptional control of HO-1 is mediated by multiple factors including NF κ B and AP-1. Both of these transcription factors are activated by free radicals generated by heme, iron, and other unrelated agents.^{5,7-10} Then what is the biological meaning of the specific induction? For some time, evidence has been accumulating to suggest that HO-1 induction is an adaptive response to cellular stresses.^{5,7,8}

In this issue of *The American Journal of Pathology*, Nath et al report experiments establishing a major protective role for HO-1 induction in acute renal failure caused by heme overload, using HO-1 gene knockout mice.¹¹ Heme overload commonly occurs when hemoglobin or myoglobin is released as a consequence of hemolysis or rhabdomyolysis. Although several cell types in the body become exposed to excessive heme when this happens, the brunt of the overload is borne by the kidney. Filtered in large amounts, heme proteins and their derivatives not

only may precipitate within tubular lumina, but also may be taken up by tubular epithelium and catabolized. These events are deleterious for the kidney and commonly lead to compromised tubular integrity and impaired glomerular and tubular function, resulting in acute renal failure (ARF). This type of ARF occurs in several clinical settings, but most commonly in crush syndrome caused by severe trauma to the extremities, first identified as an entity during the aerial bombing of London during World War II.^{12,13} Animal models of this disease caused by intramuscular injections of glycerol or intravenous infusions of hemoproteins have yielded important information on the pathogenesis of renal injury.¹³ Hypovolemia/dehydration and aciduria predispose to this type of ARF by promoting the formation of heme-containing casts, which obstruct tubular lumina. In addition, vasoconstriction and attendant decrease of glomerular filtration play important causal roles in renal failure as well. Nevertheless, the cytotoxicity of heme to epithelial cells has emerged as a major factor.

Nath and his colleagues have examined the role of HO-1 in acute renal failure caused by heme/hemoprotein overload using two experimental models.¹¹ In one model, intramuscular injection of hypertonic glycerol led to rhabdomyolysis, resulting in massive release of myoglobin from damaged muscle cells. As a result, levels of circulating heme were significantly elevated. Wild-type mice in which HO-1 was induced during the insult were able to degrade the excessive heme and sustained only mild renal insufficiency without mortality. In sharp contrast, homozygous HO-1-null mice, unable to express HO-1, developed severe renal failure with 100% mortality within 15 days of glycerol injection. Such a compelling observation was further substantiated by direct intravenous infusion of hemoglobin. Again, mutant mice without functional HO-1 gene displayed acute renal failure and marked mortality, whereas the same doses of hemoglo-

Accepted for publication March 20, 2000.

Address reprint requests to Manjeri A. Venkatachalam, M.B., B.S., Department of Pathology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229. E-mail: venkatachal@uthscsa.edu.

Heme Oxygenase in Tissue Injury and Protection

Figure 1. Heme oxygenase catalyzes the degradation of heme, resulting in the production of biliverdin, carbon monoxide (CO), and free iron in the ferrous form (Fe²⁺). In the presence of biliverdin reductase, biliverdin is rapidly converted into bilirubin. The role played by heme oxygenase in tissue pathology is determined by a delicate balance between the injurious and protective actions of heme, bilirubin, CO, and Fe²⁺.

bin were tolerated by the wild-type. Together, these results have provided clear-cut evidence of a protective role for HO-1 in ARF caused by heme or hemoprotein overload.

The finding that HO-1 induction under stressful situations is beneficial is not new. In 1992, Nath and his colleagues showed that pharmacological inhibition of HO-1 activity sensitized rat kidneys to injury.¹⁴ Moreover, exposure to large amounts of hemoproteins caused profound renal damage, whereas prior exposure of rat kidneys to subtoxic doses of hemoglobin led to the expression of HO-1 and ferritin, which conferred remarkable resistance to subsequent heme toxicity.¹⁴ In support of these observations, studies from other groups have shown that up-regulation of HO-1 through overexpression or pharmacological approaches protects a variety of cell types against injury from diverse types of insults.^{4,15–20} Moreover, recent experiments by Poss and Tönegawa have demonstrated that, in the absence of functional HO-1 gene, defensive ability of the cell against endotoxin is drastically reduced.²¹ Now, the study reported by Nath et al in this issue has further established a protective role played by HO-1 during *in vivo* acute renal failure.¹¹

With incontrovertible evidence for cytoprotection by HO-1, a tantalizing question remains only partially addressed. Could this enzyme also play a role in the development of tissue pathology? One important task for HO-1 is, of course, to clean up the excessive heme released from hemoproteins under various pathological conditions.^{5,8} Because free heme is a potent cytotoxin,^{2–4} degradation of this molecule by HO-1 appears to be an important physiological process specially designed for cellular detoxification. However, the actions of HO-1 seem to be far more complex than just that. The complexity of the actions of HO-1 stems from the pleiotropic effects of end products generated during heme catabolism (Figure 1). In the biochemical reaction catalyzed by

HO-1, the protoporphyrin is converted into biliverdin, accompanied by release of carbon monoxide and liberation of iron in the ferrous form (Fe²⁺). With biliverdin reductase, biliverdin is rapidly reduced to bilirubin, a potent physiological anti-oxidant.²² Carbon monoxide, an emerging player in signal transduction,²³ has strong vasodilatory effects^{24,25} that may ameliorate vasoconstriction and associated tissue ischemia under pathological situations including acute renal failure. On the other hand, free iron in the ferrous form (Fe²⁺) is a powerful pro-oxidant that is used in the Fenton reaction and other processes to facilitate free radical formation.^{26,27} The story does not end here, because the antioxidant bilirubin perturbs biomembranes under certain circumstances and becomes cytotoxic.^{28–30} Carbon monoxide, a vasodilator on one hand, can poison hemoproteins on the other.^{31,32} Moreover, Fe²⁺ is not always bad and can induce the expression of ferritin, a well-known cytoprotective protein that serves as an antioxidant by sequestering iron.^{33,34} Whether Fe²⁺ liberated from heme is harmful or not is therefore decided eventually by a delicate balance between two factors: the amounts of Fe²⁺ generated during heme catabolism, and the availability and/or induction of apoferritin. As a matter of fact, such a duality characterizes all of the functional aspects of HO-1. Because the final outcome of the HO-1 action is determined by the dynamics of heme clearance, biliverdin formation, Fe²⁺ release, CO generation, and ferritin induction, it is hard to predict whether HO-1 protects cells or worsens the injury in a given situation. Conceivably, it depends on conditions of the cell and levels of HO-1 expression. Zager et al³⁵ have reported experiments consistent with this line of thinking. Whereas potent renal protective effects could be demonstrated for induced HO-1 *in vivo*, acute inhibition (not induction or activation) of this enzyme exerted protection against oxidant injury of kidney proximal tubules isolated from rats with rhabdomyolysis. Because iron chelation by desferrioxamine was also similarly protective, the authors suggested that free iron liberated by HO-1 activity was a key determinant of heme cytotoxicity. Acute inhibition of HO-1 could limit the release of free iron from heme/hemoprotein and thus result in cytoprotection.³⁵

The idea that HO-1 is Janus-faced in tissue pathology is supported by recent studies of Suttner and Dennerly.³⁶ In a series of carefully controlled experiments, the investigators manipulated the levels of HO-1 within cells through overexpression using a tetracycline regulatable system. They found that low levels (two- to fivefold over that of control) of HO-1 expression are protective and moderate (10- to 15-fold) increases do not modify cell injury caused by hyperoxia, whereas high (>15-fold) levels of overexpression actually worsen the damage. Moreover, the authors demonstrated that increasing levels of HO-1 expression caused correspondingly greater elevations of intracellular free iron. Indeed, these results strongly suggest that “[r]eversal of HO-1 related cytoprotection with increased expression is due to reactive iron.”³⁶

Thus, HO-1 induction to appropriate levels is beneficial for cell survival, whereas too much HO-1 can have op-

posite, adverse effects. In the experimental models of acute renal failure used by Nath et al,¹¹ HO-1 expression was shown to be two- to fourfold of that in control, well within the protective range.³⁶ In this context, it is prudent to re-emphasize the roles played by (heme) protein-related tubular obstruction and renal vasoconstriction in myohemoglobinuric ARF. Protection afforded by HO-1 may depend to a large extent simply on greater clearance of heme systemically by induced HO-1, and the removal of accumulating heme from tubular lumina by catabolism, thus ameliorating the severity of tubular obstruction. Moreover, by removing heme from the kidney, HO-1 may have major beneficial effects on the renal vasculature. On the one hand, generation of CO will promote vasodilation; on the other, removal of heme will result in decreased scavenging of NO by heme,³⁷⁻³⁹ increased vascular NO levels, and amelioration of vasoconstriction. In contrast, lack of the enzyme in HO-1^{-/-} mice will lead to increasing tissue heme levels and worsen renal failure by increasing the severity of tubular obstruction and vasoconstriction. The complex interplay of the "yin and yang" of heme oxygenase and the importance of defining the context in which the enzyme is induced cannot be overstated. The bewildering duality of its eventual effects is further illustrated by the fact that heme oxygenase activity can down-regulate or inactivate nitric oxide synthase (NOS).⁵ For example, CO and Fe²⁺ produced as a result of heme oxygenase activity can inactivate NOS and decrease synthesis of the enzyme, respectively.⁵

In view of the demonstration by Nath et al¹¹ that HO-1 induction during rhabdomyolysis is within the protective range,³⁶ the question arises whether HO-1 induction is ultimately beneficial or protective in other clinically relevant circumstances. A wide range of HO-1 induction has been documented in diverse pathophysiological conditions that are potentially injurious.⁴⁰⁻⁵¹ Therefore, to classify HO-1 simply as a protective protein seems to be an oversimplification. Nevertheless, identification of the beneficial or adverse effects of HO-1 in a given clinical setting is certainly of significance, because it holds great promise to reduce tissue pathology through manipulation of HO-1 expression using genetic or pharmacological strategies.

References

- Ponka P: Cell biology of heme. *Am J Med Sci* 1999, 318:241-256
- Balla J, Jacob HS, Balla G, Nath K, Eaton JW, Vercellotti GM: Endothelial-cell heme uptake from heme proteins: induction of sensitization and desensitization to oxidant damage. *Proc Natl Acad Sci USA* 1993, 90:9285-9289
- Nath KA, Grande JP, Croatt AJ, Likely S, Hebbel RP, Enright H: Intracellular targets in heme protein-induced renal injury. *Kidney Int* 1998, 53:100-111
- Abraham NG, Lavrovsky Y, Schwartzman ML, Stoltz RA, Levere RD, Gerritsen ME, Shibahara S, Kappas A: Transfection of the human heme oxygenase gene into rabbit coronary microvessel endothelial cells: protective effect against heme and hemoglobin toxicity. *Proc Natl Acad Sci USA* 1995, 92:6798-6802
- Maines MD: The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 1997, 37:517-554
- McCoubrey WK, Jr., Huang TJ, Maines MD: Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. *Eur J Biochem* 1997, 247:725-732
- Elbirt KK, Bonkovsky HL: Heme oxygenase: recent advances in understanding its regulation and role. *Proc Assoc Am Physicians* 1999, 111:438-447
- Choi AM, Alam J: Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am J Respir Cell Mol Biol* 1996, 15:9-19
- Lavrovsky Y, Schwartzman ML, Levere RD, Kappas A, Abraham NG: Identification of binding sites for transcription factors NF-kappa B and AP-2 in the promoter region of the human heme oxygenase 1 gene. *Proc Natl Acad Sci USA* 1994, 91:5987-5991
- Lavrovsky Y, Song CS, Chatterjee B, Roy AK: Age-dependent increase of heme oxygenase-1 gene expression in the liver mediated by NFkB. *Mech Ageing Dev* 2000 114:49-60
- Nath KA, Haggard JJ, Croatt AJ, Grande JP, Poss KD, Alam J: The indispensability of heme oxygenase-1 (HO-1) in protecting against acute heme protein-induced toxicity in vivo. *Am J Pathol* 2000, 156: 1527-1535
- Better OS, Rubinstein I, Winaver J: Recent insights into the pathogenesis and early management of the crush syndrome. *Semin Nephrol* 1992, 12:217-222
- Zager RA: Rhabdomyolysis and myohemoglobinuric acute renal failure. *Kidney Int* 1996, 49:314-326
- Nath KA, Balla G, Vercellotti GM, Balla J, Jacob HS, Levitt MD, Rosenberg ME: Induction of heme oxygenase is a rapid, protective response in rhabdomyolysis in the rat. *J Clin Invest* 1992, 90:267-270
- Dennery PA, Sridhar KJ, Lee CS, Wong HE, Shokoohi V, Rodgers PA, Spitz DR: Heme oxygenase-mediated resistance to oxygen toxicity in hamster fibroblasts. *J Biol Chem* 1997, 272:14937-14942
- Lee PJ, Alam J, Wiegand GW, Choi AM: Overexpression of heme oxygenase-1 in human pulmonary epithelial cells results in cell growth arrest and increased resistance to hyperoxia. *Proc Natl Acad Sci USA* 1996, 93:10393-10398
- Panahian N, Yoshiura M, Maines MD: Overexpression of heme oxygenase-1 is neuroprotective in a model of permanent middle cerebral artery occlusion in transgenic mice. *J Neurochem* 1999, 72:1187-1203
- Amersi F, Buelow R, Kato H, Ke B, Coito AJ, Shen XD, Zhao D, Zaky J, Melinek J, Lassman CR, Kolls JK, Alam J, Ritter T, Volk HD, Farmer DG, Ghobrial RM, Busuttill RW, Kupiec-Weglinski JW: Upregulation of heme oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. *J Clin Invest* 1999, 104:1631-1639
- Ferris CD, Jaffrey SR, Sawa A, Takahashi M, Brady SD, Barrow RK, Tysoe SA, Wolosker H, Baranano DE, Dore S, Poss KD, Snyder SH: Haem oxygenase-1 prevents cell death by regulating cellular iron. *Nat Cell Biol* 1999, 1:152-157
- Soares MP, Lin Y, Anrather J, Csizmadia E, Takigami K, Sato K, Grey ST, Colvin RB, Choi AM, Poss KD, Bach FH: Expression of heme oxygenase-1 can determine cardiac xenograft survival. *Nat Med* 1998, 4:1073-1077
- Poss KD, Tonegawa S: Reduced stress defense in heme oxygenase 1-deficient cells. *Proc Natl Acad Sci USA* 1997, 94:10925-10930
- Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN: Bilirubin is an antioxidant of possible physiological importance. *Science* 1987, 235:1043-1046
- Snyder SH, Jaffrey SR, Zakhary R: Nitric oxide and carbon monoxide: parallel roles as neural messengers. *Brain Res Brain Res Rev* 1998, 26:167-175
- Coceani F: Carbon monoxide and dilation of blood vessels. *Science* 1993, 260:739
- Leffler CW, Nasjletti A, Yu C, Johnson RA, Fedinec AL, Walker N: Carbon monoxide and cerebral microvascular tone in newborn pigs. *Am J Physiol* 1999, 276:H1641-H1646
- Fridovich I: Superoxide anion radical (O₂⁻), superoxide dismutases, and related matters. *J Biol Chem* 1997, 272:18515-18517
- Yu BP: Cellular defenses against damage from reactive oxygen species. *Physiol Rev* 1994, 74:139-162
- Vazquez J, Garcia-Calvo M, Valdivieso F, Mayor F, Mayor F Jr: Interaction of bilirubin with the synaptosomal plasma membrane. *J Biol Chem* 1988, 263:1255-1265
- Leonard M, Noy N, Zakim D: The interactions of bilirubin with model and biological membranes. *J Biol Chem* 1989, 264:5648-5652

30. Mireles LC, Lum MA, Dennerly PA: Antioxidant and cytotoxic effects of bilirubin on neonatal erythrocytes. *Pediatr Res* 1999, 45:355-362
31. Ernst A, Zibrak JD: Carbon monoxide poisoning. *N Engl J Med* 1998, 339:1603-1608
32. Hawkins M: Carbon monoxide poisoning. *Eur J Anaesthesiol* 1999, 16:585-589
33. Balla G, Jacob HS, Balla J, Rosenberg M, Nath K, Apple F, Eaton JW, Vercellotti GM: Ferritin: a cytoprotective antioxidant strategem of endothelium. *J Biol Chem* 1992, 267:18148-18153
34. Harrison PM, Arosio P: The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim Biophys Acta* 1996, 1275:161-203
35. Zager RA, Burkhardt KM, Conrad DS, Gmur DJ: Iron, heme oxygenase, and glutathione: effects on myohemoglobinuric proximal tubular injury. *Kidney Int* 1995, 48:1624-1634
36. Suttner DM, Dennerly PA: Reversal of HO-1 related cytoprotection with increased expression is due to reactive iron. *FASEB J* 1999, 13:1800-1809
37. Braughler JM, Mittal CK, Murad F: Effects of thiols, sugars, and proteins on nitric oxide activation of guanylate cyclase. *J Biol Chem* 1979, 254:12450-12454
38. Furchgott RF, Jothianandan D: Endothelium-dependent and -independent vasodilation involving cyclic GMP: relaxation induced by nitric oxide, carbon monoxide and light. *Blood Vessels* 1991, 28: 52-61
39. Sharma VS, Traylor TG, Gardiner R, Mizukami H: Reaction of nitric oxide with heme proteins and model compounds of hemoglobin. *Biochemistry* 1987, 26:3837-3843
40. Ewing JF, Maines MD: Rapid induction of heme oxygenase 1 mRNA and protein by hyperthermia in rat brain: heme oxygenase 2 is not a heat shock protein. *Proc Natl Acad Sci USA* 1991, 88:5364-5368
41. Rizzardini M, Terao M, Falciani F, Cantoni L: Cytokine induction of haem oxygenase mRNA in mouse liver: interleukin 1 transcriptionally activates the haem oxygenase gene. *Biochem J* 1993, 290:343-347
42. Kutty RK, Kutty G, Wiggert B, Chader GJ, Darrow RM, Organisciak DT: Induction of heme oxygenase 1 in the retina by intense visible light: suppression by the antioxidant dimethylthiourea. *Proc Natl Acad Sci USA* 1995, 92:1177-1181
43. Premkumar DR, Smith MA, Richey PL, Petersen RB, Castellani R, Kutty RK, Wiggert B, Perry G, Kalaria RN: Induction of heme oxygenase-1 mRNA and protein in neocortex and cerebral vessels in Alzheimer's disease. *J Neurochem* 1995, 65:1399-1402
44. Willis D, Moore AR, Frederick R, Willoughby DA: Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med* 1996, 2:87-90
45. Vogt BA, Shanley TP, Croatt A, Alam J, Johnson KJ, Nath KA: Glomerular inflammation induces resistance to tubular injury in the rat: a novel form of acquired, heme oxygenase-dependent resistance to renal injury. *J Clin Invest* 1996, 98:2139-2145
46. Yet SF, Pellacani A, Patterson C, Tan L, Folta SC, Foster L, Lee WS, Hsieh CM, Perrella MA: Induction of heme oxygenase-1 expression in vascular smooth muscle cells: a link to endotoxic shock. *J Biol Chem* 1997, 272:4295-4301
47. Vile GF, Basu-Modak S, Waltner C, Tyrrell RM: Heme oxygenase 1 mediates an adaptive response to oxidative stress in human skin fibroblasts. *Proc Natl Acad Sci USA* 1994, 91:2607-2610
48. Acevedo CH, Ahmed A: Hemeoxygenase-1 inhibits human myometrial contractility via carbon monoxide and is upregulated by progesterone during pregnancy. *J Clin Invest* 1998, 101:949-955
49. Mautes AE, Kim DH, Sharp FR, Panter S, Sato M, Maida N, Bergeron M, Guenther K, Noble LJ: Induction of heme oxygenase-1 (HO-1) in the contused spinal cord of the rat. *Brain Res* 1998, 795:17-24
50. Reeve VE, Tyrrell RM: Heme oxygenase induction mediates the photoimmunoprotective activity of UVA radiation in the mouse. *Proc Natl Acad Sci USA* 1999, 96:9317-9321
51. Doi K, Akaike T, Fujii S, Tanaka S, Ikebe N, Beppu T, Shibahara S, Ogawa M, Maeda H: Induction of haem oxygenase-1 nitric oxide and ischaemia in experimental solid tumours and implications for tumour growth. *Br J Cancer* 1999, 80:1945-1954