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Forum Editorial

Heme Oxygenase-1: Past, Present, and Future

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IRON is essential for life—of that there is no doubt. Iron, however, has not always been a "user-friendly" molecule. With the appearance of photosynthetic organisms and the concomitant release of the "environmental pollutant" dioxygen, iron-dependent organisms were forced to develop efficient strategies for the maintenance, storage, and utilization of iron in an oxidizing environment that tended to generate a highly insoluble form of this metal, Fe(III). Moreover, Fe(III) can undergo redox cycling to Fe(II) in the course of which toxic reactive oxygen intermediates are generated. In view of these considerations, it is thus not surprising that in most, if not all, organisms, the majority of cellular iron exists primarily as the protoporphyrin chelate, heme. In humans, for instance, it is estimated that 75–80% of the total body iron is in the form of heme bound to proteins, and the majority of the remainder is complexed with the iron storage protein, ferritin. As heme is the prosthetic group of numerous proteins, including hemoglobin, cytochromes, and catalase, it is essential for diverse biological processes such as oxygen transport, electron transfer, energy production, and biotransformation of xenobiotics.

Because of its critical role in many biological reactions and the fact that uncomplexed, "free" heme is highly toxic to cellular structures, humans and other organisms have evolved highly efficient, compartmentalized, and regulated mechanisms for the synthesis, sequestration/transport, and catabolism of this molecule. In mammalian cells, heme catabolism is generally considered a two-step process. In the initial, rate-limiting reaction, microsomal heme oxygenase (HO) catalyzes the oxygen-dependent cleavage of the tetrapyrrole ring to generate biliverdin with the release of carbon monoxide (CO) and the iron molecule (Rx. 1). In the subsequent reaction (Rx. 2), biliverdin is reduced to bilirubin by the action of cytosolic biliverdin reductase. (Reactants and products are not presented in stoichiometric amounts.)

$$\text{Heme} + O_2 + \text{NADPH} \rightarrow \text{Biliverdin} + \text{CO} + \text{Fe} + \text{NADP} \quad \text{(Rx. 1)}$$

$$\text{Biliverdin} + \text{NADPH} \rightarrow \text{Bilirubin} + \text{NADP} \quad \text{(Rx. 2)}$$

The discovery of HO is generally attributed to Rudi Schmid and colleagues who, in 1968, described the enzymatic conversion of heme to bilirubin using microsome fractions from various tissues, including the liver and spleen (16, 17). It is now clear that the enzyme activity initially detected in the liver represents the combined activities of at least two distinct isoforms, the substrate-inducible HO-1 and the non-inducible HO-2. Most of the subsequent work in this field has focused on the inducible isoform. As would be expected of a newly discovered enzyme, there was a slow, but gradual, increase in the number of studies on "heme oxygenase," reaching a plateau between the years of 1982 and 1992 (Fig. 1). This maturation presumably reflected the then accepted paradigm of HO enzymes as simply the control point for the essential, but unassuming, task of heme degradation. During most of this period, it is unlikely that many would have predicted that the next decade would bring a dramatic upsurge in interest in the heme degradation pathway with revolutionary new paradigms of HO function.

Arguably, this renewed interest in HO, particularly HO-1, can be traced to four different experimental observations. (a) In a series of studies in the late 1980s Ames, Stocker, and their colleagues demonstrated that both biliverdin and bilirubin possess potent antioxidant activities (15 and references therein). (b) Similarly, in the early 1990s, Marks *et al.* (9) and Snyder and colleagues (18) suggested that another product of heme catabolism, CO, may mediate physiological functions, including vasorelaxation and neurotransmission. This sudden rehabilitation of molecules previously considered to be biological waste products into useful bioactive agents profoundly influenced our view of the reaction catalyzed by HO enzymes; this reaction constituted not only a catabolic pathway for the disposal of heme, but also an anabolic pathway for the elaboration of bioactive molecules (bile pigments, CO) with multiple, and predominantly beneficial, physiological effects. (c) Soon after the initial characterization of HO activity, several investigators reported that this activity could be stimulated by various nonheme agents, including endotoxin, heavy metals, and hormones. Subsequent studies would demonstrate that this induction represented *de novo*

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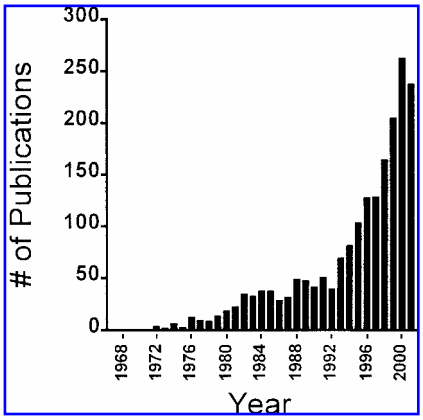

FIG. 1. Annual publications referring to heme oxygenase. Based on a PubMed search using the term "heme oxygenase."

synthesis of HO-1. Over the years, as the list of HO-1 inducers has expanded (8), so has the effort to define a unifying mechanism to explain the induction of HO-1 by chemically and structurally diverse agents. One initial speculation centered on the ability of some of these agents to mobilize a putative intracellular heme pool, but it readily became evident that many of these inducers functioned via heme-independent mechanisms. In 1991, Tyrrell and his colleagues (1) noted that many, if not all, HO-1 inducers could promote a cellular prooxidant state and that induction of HO-1 represented a general response to oxidant stress. In this scenario, cellular oxidative stress, resulting from either increased production of reactive oxygen species or decreased levels of intracellular reductants, would be the common effector system for the various HO-1 inducers. Possibly reflecting on the studies of Ames and Stocker, Tyrrell and colleagues further concluded that "although functional evidence for a protective role is still lacking . . . the properties of the enzyme itself encourage the hypothesis that the" induction of HO-1 "reflects a powerful mechanism by which the prooxidant state of cells can be transiently lowered in order to avoid damage during a sustained oxidative stress." (d) The "functional evidence" that Tyrrell and colleagues sought was soon forthcoming. The following year, Nath *et al.* (11) demonstrated that prior induction of HO-1 protected rats from renal failure and mortality resulting from glycerol-induced rhabdomyolysis; the opposite effect was observed upon inhibition of HO activity. Subsequent studies by multiple investigators have confirmed the protective function of HO-1 induction and activity during injury initiated by both heme and nonheme insults. In the latter cases, it is assumed, although not necessarily proven, that the cellular disruption caused by nonheme toxicants also promotes release of, or access to, sufficient amounts of substrate for the induced HO-1 enzyme.

Together, the seminal studies noted above not only have led to the increased interest in "heme oxygenase" highlighted in Fig. 1, but have irreversibly altered our conception of the HO-1 enzyme. Although HO-1 is still known to catalyze only one enzymatic reaction—the oxidative cleavage of heme—the cellular and physiological consequences of this reaction are considerably more significant. Because of this reaction, HO-1 manifests antioxidant, antiinflammatory, and antiapoptotic activities, modulates inter- and intracellular signaling mechanisms, and ultimately participates in the more general process of cellular homeostasis in response to injury. For instance, the regulation of the prevailing levels of "reactive" iron is one aspect of cellular homeostasis in which HO-1 has received considerable attention: while acutely increasing cellular levels, induction of HO-1 ultimately restrains the elevation in intracellular levels of reactive iron as HO-1 facilitates the synthesis of iron-binding proteins and the expression of iron-exporting proteins, the latter serving to transfer iron out of cells. Thus, HO-1 may exert antioxidant properties not only by degrading heme and generating bile pigments, but also by facilitating the cellular storage or cellular export of iron. Given the widespread recognition of the involvement of oxidative stress in a plethora of human pathologies along with the documented pleiotropic effects of CO, it is not surprising that the HO-1 field has expanded into studies of multiple and diverse physiological (*e.g.,* cellular proliferation, cell signaling, apoptosis) and pathophysiological (*e.g.,* atherosclerosis, cancer, organ transplantation and preservation) processes. This expansion, in turn, has produced a similar diversity in scientific ideas and endeavors, many of which are evident in the review and original articles that collectively form the current **Forum.**

Angela Wilks reviews the current state of knowledge of the enzymatic mechanism and three-dimensional structure of HO-1, including a comparison between the structures of mammalian and bacterial enzymes (19). This review is a useful reminder that, although much of the interest in HO-1 is focused on mammalian systems, chelation of iron into the porphyrin ring is an old and ubiquitous strategy, and HOs by necessity are widely distributed along the many branches of the evolutionary tree. Given the current interest in sequencing of the human genome and identification of diagnostic polymorphisms, another timely subject is addressed by Shibahara and colleagues (14), who scrutinize the interspecies and interindividual variations in the *ho-1* gene and its regulation, including stress-induced repression of HO-1 in some human cells. Such variations in HO-1 expression have been largely ignored, but may be of particular significance to the development of some human diseases. The down-modulation of the *ho-1* gene in certain cellular context is only one paradox of the HO-1 regulatory system. Another apparent contradiction, induction of HO-1 expression by both high oxygen concentration (hyperoxia) and low oxygen tension (hypoxia), is reviewed by Ryter and Choi (12). The authors examine the mechanistic basis for such induction, including the role of reactive oxygen species in *ho-1* gene activation by both hyperoxia and hypoxia.

Given the novelty and limited examples of endogenously generated, bioactive gaseous molecules, CO has received considerable attention as of late. Accumulating evidence indicates that CO modulates multiple cellular and physiological processes, but its mechanism of action is not well understood. Presumably, these effects are initiated by the binding of CO to the iron atom of hemoproteins or iron-sulfur proteins (9). One widely accepted CO target is soluble guanylate cyclase (sGC), activation of which leads to increased production of the second messenger cyclic GMP. Kajimura *et al.* (7) discuss

the potential interaction between CO and other "receptors," such as cytochrome P450 monooxygenases, resulting in the activation of different signaling pathways. The authors also elaborate on their recent hypothesis that, in contrast to its conventional role as an activator of sGC, CO may function as a partial antagonist of this enzyme in the presence of high levels of nitric oxide (NO), a gaseous molecule generated by the action of nitric oxide synthases (NOS). A more detailed analysis of the interplay between the two dominant gaseous signaling systems, CO/HO and NO/NOS, and the effects of nitrosative stress on HO-1 expression is presented by Motterlini and colleagues (10).

At the risk of overgeneralization, current research on HO-1 can be divided into two very broad categories: those related to the regulatory mechanisms of HO-1 expression (in particular, its induction) and those related to the functional consequence of such expression. Both types of research are represented in the present **Forum.** Induction of HO-1 expression is regulated primarily at the level of gene transcription, and studies in the past decade have identified various *cis*-elements and transcription factors responsible for inducer-mediated *ho-1* gene activation (2). The signal transduction pathways utilized for *ho-1* gene activation, however, are less well characterized. Recent studies have consistently demonstrated a positive role for one or more of the mitogen-activated protein kinase (MAPK) pathways in HO-1 induction by several agents such as arsenite, heavy metals, and NO. For instance, p38 MAPK signaling, but not the extracellular signal-regulated kinase (ERK) pathway, appears to be necessary for HO-1 induction by hypoxia in cardiomyocytes (6). In stark contrast, the article by Ryter *et al.* (13) in this issue shows that both the ERK and p38 MAPK pathways negatively influence hypoxia-stimulated HO-1 expression in vascular endothelial and smooth muscle cells. This finding suggests that the role of MAPKs in *ho-1* gene activation not only is inducer-dependent, but may also vary with cellular context.

Ejima *et al.* (3) examine a different type of signaling pathway, namely, the thioredoxin/thioredoxin reductase system, in *ho-1* gene regulation. This system is responsible for maintaining intracellular proteins in a reduced state. Because thioredoxin can reactivate oxidatively inactivated transcription factors such as AP-1, it may facilitate *ho-1* gene induction under oxidizing conditions. These investigators provide correlative evidence for a role of the thioredoxin system in lipopolysaccharide-induced HO-1 expression in macrophages. More importantly, they directly demonstrate, for the first time, that ectopic overexpression of thioredoxin stimulates HO-1 production in these cells.

With regard to HO-1 function, Goto *et al.* (4) describe the development of monocrotaline-induced pulmonary hypertension and inflammation in mice, a model in which induction of HO-1 expression and enzymatic activity provides a counterbalance to the inflammatory and hypertensive responses. Although similar experimental formats have been described using other animals, the development of a murine model will permit analysis of the antiinflammatory function of HO-1 in different genetic backgrounds (*i.e.,* using genetically altered mice) and of the interrelationship between HO-1 and other gene products in complex cellular and physiological responses. One such relationship is illustrated in the article by Jozkowicz *et al.* (5), who present their novel observation that increased synthesis of vascular endothelial growth factor (VEGF) in response to 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 is dependent on the induction and activity of HO-1. Thus, VEGF joins a growing list of proteins, including ferritin, tumor necrosis factor-α and other cytokines, and the cell cycle-inhibitory protein p21, whose expression is modulated by HO-1 activity.

It would appear that interest in "heme oxygenase" is in the early stage of another plateau (Fig. 1). Whether this represents the final maturation of this field or a temporary holding position prior to another paradigm shift remains to be seen. Regardless of the ultimate scenario, the intense activity in the past 10 years has generated as many questions as answers, and much remains to be done. A particularly relevant question is whether the knowledge gained during this period can be translated into practical therapies for one or more human diseases.

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ABBREVIATIONS

CO, carbon monoxide; ERK, extracellular signal-regulated kinase; HO, heme oxygenase; MAPK, mitogen-activated protein kinase; NO, nitric oxide; NOS, nitric oxide synthase; sGC, soluble guanylate cyclase; VEGF, vascular endothelial growth factor.

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