Heme Oxygenase-1: A Fundamental Guardian Against Oxidative Tissue Injuries in Acute Inflammation

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Abstract: Free heme contributes as a major threat to the oxidative tissue injuries because it catalyzes the formation of reactive oxygen species. When free heme concentration is increased, it results in the induction of heme oxygenase-1 (HO-1), which then breaks free heme down. As such, HO-1 plays a pivotal role in the protection of tissues from oxidative injuries.

Key Words: Carbon monoxide, heme, heme oxygenase-1, inflammation, liver injury, oxidative stress, renal injury, sepsis.

INTRODUCTION

Heme is ferrous protoporphyrin-IX that is the prosthetic group of hemoproteins, such as hemoglobin, myoglobin and cytochromes that are of vital importance [1, 2]. In contrast, "free heme", a protein-unbound heme, that is either just synthesized but yet not incorporated into hemoproteins [3], or that is released from hemoprotein under oxidative conditions, is highly toxic, since it catalyzes the production of reactive oxygen species (ROS) [1, 2, 4]. In order to cope with this problem, the body is equipped with various defense mechanism(s) against an excessive amount of "free heme" concentrations. Heme oxygenase (HO) is one of the key players in the defense mechanism, and plays a fundamental role against the free-heme mediated oxidative process [5-8].

HO is the rate-limiting enzyme in the degradation of heme, and oxidatively cleaves heme to yield one molecule each of iron, carbon monoxide (CO), and biliverdin IXα. Biliverdin IXα is then reduced to bilirubin IXα by biliverdin reductase (BVR) (Fig. **1**) [9]. Among the three HO isoforms, HO-1 and HO-2 have a *bona fide* HO activity, while HO-3 has no enzymatic activity at all [10, 11]. HO-1 is inducible in response to various stimuli [9], while HO-2 is constitutively expressed and not inducible [10]. HO-3 has recently been shown to be a pseudogene product from the *ho-2* gene [12]. HO-1 is induced in various cell types not only by its substrate heme itself but also by a vast array of stressful stimuli including oxidative tissue injuries associated with acute illness, such as acute liver injury, acute renal injury, or septic tissue injury [5-8].

Until a few years ago, two metabolites of heme produced by HO-1 or HO-2, i.e., biliverdin IXα and CO, used to be thought useless or toxic, but recent evidence indicates that they have significant and useful biological properties, such as anti-oxidant, anti-inflammatory, or anti-apoptotic activities [13, 14]. Thus, in addition to the removal of free heme, i.e., a potent pro-oxidant, HO-1 induction results in the production of anti-oxidant molecules which act as a member of the protective response, and contribute to the suppression of oxidative tissue injuries. The importance of HO-1 in the protection from oxidant stresses is further substantiated by findings in mutant mice with *ho-1* knockout and in a human patient with inherited HO-1 deficiency [15, 16]. For example, the absence of HO-1 in a patient with hereditary HO-1 deficiency was associated with an abnormally elevated serum heme concentrations (~ 0.5 mM), and various intensive oxidative as well as massive inflammatory complications [16]. The strong adaptive response of HO-1 to various stimuli suggests an entirely new paradigm for this enzyme to play a significant role in the protection of inflammatory processes, especially that of free heme-mediated oxidative tissue injuries, and HO-1 should now be recognized as a fundamental guardian against free heme-mediated oxidative tissue injuries.

1. SUBCELLULAR LOCALIZATION OF HO-1

The metabolism of heme is catalyzed by a sequence of three enzymatic reactions, involving NADPH-cytochrome P450 reductase (CR), HO and BVR. The initial step in this sequence is the formation of a ferric-heme-HO complex, which is then oxidized by a reducing equivalent provided by CR in the presence of NADPH. HO is the enzyme that catalyzes the regiospecific cleavage of the α-methene bridge of heme to yield iron, biliverdin IXα, and CO, and is the rate-limiting step in the entire sequence [9]. Importantly, HO can form an equimolar complex with CR [17], suggesting that the heme cleavage reaction may proceed in a very efficient manner within the binary complex. Biliverdin IXα is reduced to bilirubin IXα, the major bile pigment, by cytosolic BVR. Enzyme kinetic analysis suggests that BVR may also interact with the CR-HO complex [17]. Thus, biliverdin IXα, formed by the CR-HO complex, might also be converted to bilirubin IXα without leaving the enzyme complex in the endoplasmic reticulum.

HO enzymes have been recognized as endoplasmic reticulum-associated proteins since their initial characterization in 1969 [18]. However, recent studies have suggested that HO-1 protein or its activity can be demonstrated in other

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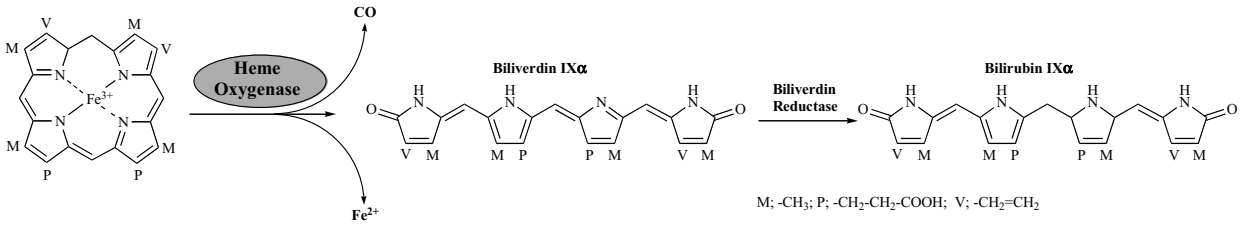

Fig. (1). Catalysis of heme by heme oxygenase.

Heme oxygenase catalyzes the oxidative cleavage of heme to yield equimolar amounts of CO, iron (Fe^{2+}), and biliverdin. Biliverdin is subsequently converted to bilirubin through the action of biliverdin reductase.

subcellular organelles beside endoplasmic reticulum. Recently, it has been reported that, in pulmonary endothelial cells after stimulation with inducers of HO-1, HO-1 as well as CR and BVR were functionally demonstrated in part in association with specialized plasma membrane caveolae [19]. This finding suggests that heme degradation in the lung may also occur in the plasma membrane caveolae. Most recently, functional HO-1 protein has also been shown to localize in the rat liver mitochondria [20]. Namely, treatment of rats with hemin increased HO-1 targeting to the inner mitochondrial membrane, which was also found to contain BVR, supporting that both enzymes are in the same compartment [20]. Moreover, HO-1 induction in mitochondria led to the modulation of mitochondrial heme and hemoproteins levels such as cytochrome oxidase subunit I and mitochondria-targeted nitric oxide synthase, and resulted in changes in mitochondrial oxygen uptake and ROS production [20]. Nuclear localization of HO-1 has also been reported in astroglial cells stimulated with glutamate [21]. In addition, nuclear localization of BVR has been reported in response to endotoxin or bromobenzene treatment in the rat kidney [22], suggesting the possible functional compartmentalization of the heme degrading system in the nucleus in certain organs. All these findings suggest that, in addition to the endoplasmic reticulum, HO-1 localization in other subcellular organelles may also occur. How these compartmentalizations of HO-1 influences the fine tuning of cellular heme concentration remains yet elusive, and further studies are necessary to clarify this question.

2. HO-1 INDUCTION AND ITS PROTECTIVE ROLE IN FREE HEME-MEDIATED OXIDATIVE TISSUE INJURUES IN ACUTE ILLNESS

Oxidative tissue injuries can frequently be found in association with increased free heme concentrations [2]. HO-1 is also markedly induced either by treatment with hemin (free heme), or as a result of acute inflammation, suggesting a possible role of HO-1 in the protection of oxidative inflammatory stress [2, 5-8]. This question has been examined in several animal models of oxidative tissue injuries.

2.1. HO-1 Induction is a Protective Response in the Oxidative Liver Injury

The liver is the central organ that detoxifies endogenously produced waste products, and exogenously derived toxins or drugs. The hepatic drug metabolizing enzyme cytochrome P450 (CYP) plays a key role in this detoxifying process. This process itself often generates reactive intermediate(s) [23] which then degrades CYP leading to the release of free heme [24, 25] (Fig. **2**). As a result, increased free heme concentration in hepatocytes on one hand leads to the production of ROS and tissue injuries, but on the other hand, results in a marked induction of HO-1 which protects liver cells from further damages by the free heme-mediated toxicity [6-8].

2.1.1. Protection of Acute Liver Injury Following Halothane Exposure by HO-1 Induction

Oxidative liver injuries can occur in association with inhalation of certain volatile anesthetics [26]. For example, halothane is metabolized by a reductive pathway under a hypoxic condition, to yield a free radical intermediate(s) which initiates lipid peroxidation and intensive hepatic injury [27]. Halothane inhalation in rats was shown to cause liver injury when it was administered together with phenobarbital (PB). This finding suggests that increased turnover of CYP induced by PB is necessary in the halothane-mediated liver injury [25]. The halothane-hypoxia exposure in PB-pretreated rats also resulted in a rapid increase in hepatic intracellular free heme, which was most likely derived from the destruction of PB-induced CYP. The halothane-hypoxia treatment also accompanied the induction of HO-1 mRNA and its enzyme activity in hepatocytes around the central vein [25]. Pretreatment of these rats with hemin, which induced HO-1, significantly attenuated the halothane-induced hepatic injury, as judged by suppression of halothane-induced alanine transaminase (ALT) activity, and by normalization of liver histology [25]. In contrast, inhibition of HO activity by the administration of tin-mesoporphyrin (SnMP), a specific competitive inhibitor of HO activity, entirely abolished the beneficial effect of hemin [6]. HO-1 induction thus plays an important role in the protection of the hepatic injury due to oxidative damages caused by halothane-hypoxia exposure.

2.1.2. Amelioration of the Carbon Tetrachloride-Induced Hepatic Injury by Liver-Specific HO-1 Induction

Treatment of animals with carbon tetrachloride (CCl_4) is known to cause severe hepatic injury [24]. CCl_4 is reductively metabolized by hepatic CYP with the production of a reactive intermediate that catalyzes the production of lipid peroxides, and the breakdown of cellular membranes [23]. CCl_4 treatment has also been shown to accompany a rapid increase in microsomal heme levels which was likely due to the destruction of hepatic CYP, and to result in marked HO-1 induction in hepatocytes [24]. Inhibition of HO activity by

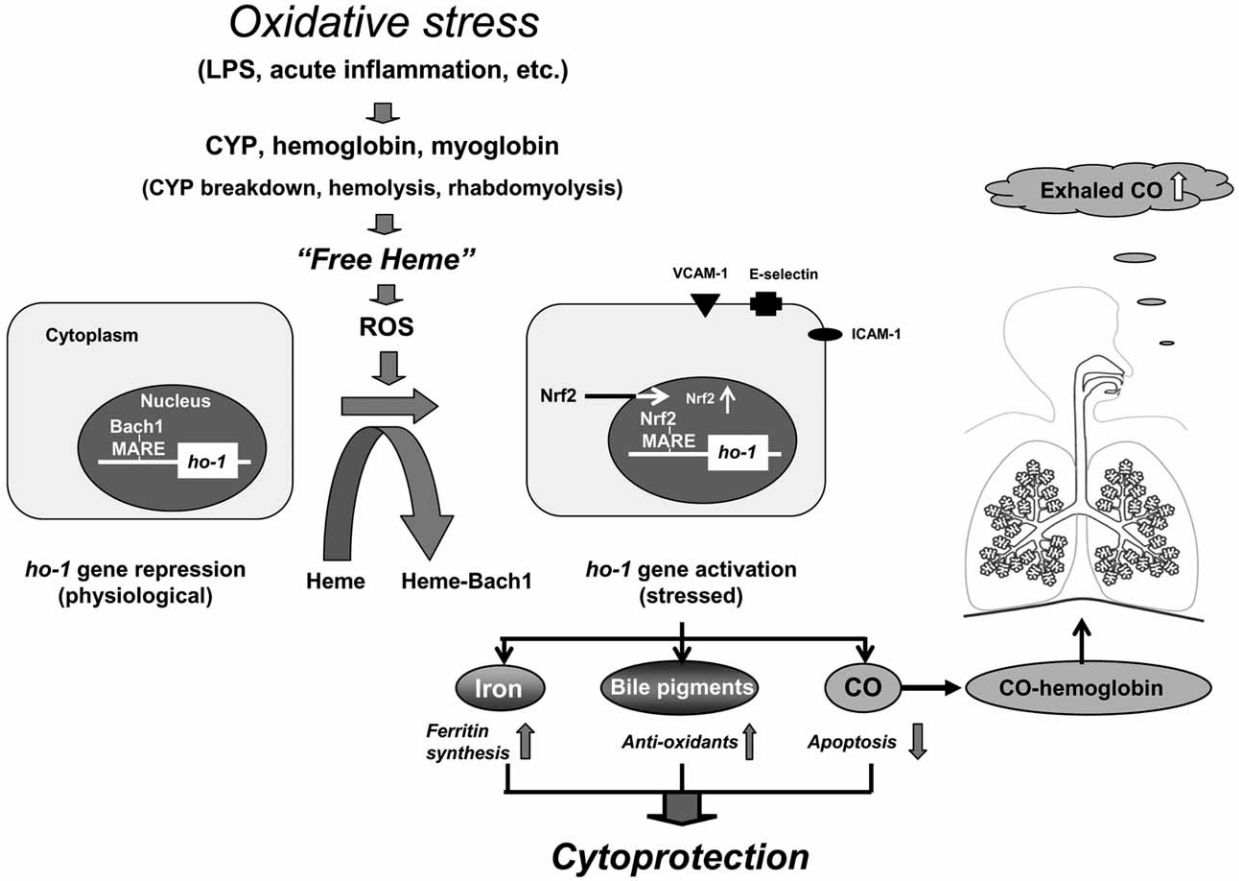

Fig. (2). Consequences of heme catabolism in acute illness.

Oxidative stresses in acute illness, such as acute liver injury, acute renal injury or septic tissue injury, result in the production of free heme, which is likely released from cytochrome P450 (CYP), or from other hemoproteins. Free heme is then involved in the generation of reactive oxygen species (ROS) further to increase oxidative stress. Free heme also binds with Bach1, the transcriptional repressor of *ho-1*, and allows its displacement from MARE sequence in the *ho-1* promoter. This in turn allows Nrf2 binding with MARE, and induces transcriptional activation of the *ho-1* gene. Heme is also known to stimulate nuclear translocation of Nrf2. As HO-1 expression is known to be principally regulated at the transcriptional level, this sequence of events results in the increase in HO-1 protein and its enzymatic activity. HO-1 induction may take place not only in endoplasmic reticulum but also in plasma membrane caveolae and mitochondria in certain tissues or organs. Increased HO-1 activity then metabolizes free heme to iron, CO and biliverdin IXα. Iron is directly sequestered and inactivated by co-induced ferritin. Biliverdin IXα is rapidly converted to bilirubin IXα by biliverdin reductase, and both bile pigments serve as a major anti-oxidant. CO can suppress apoptosis of endothelial cells *via* the activation of p38 MAPK. Thus, all these metabolites of the HO reaction act as a member of the adaptive response and confer protection on cells from further oxidative injuries. On the other hand, CO synthesized through the HO reaction diffuses out of cells, enters the blood to form CO-hemoglobin and is transported to the lung where it is excreted into ambient air, leading to the increase in the concentrations of CO in exhaled air which is observed in various inflammatory disorders, such as asthma and sepsis.

pretreatment of animals with SnMP resulted in a sustained increase in microsomal heme concentration, as well as in the aggravation of hepatic injury and exacerbation of tissue inflammation [24]. Thus, HO-1 induction and an intact HO activity are critical in the protection of liver cells from an oxidative injury caused by CCl_4, while the ablation of HO activity aggravates the CCl_4-induced toxicity. These findings point to the fact that free heme derived from CYP plays a significant role in the oxidative tissue injuries, and that associated induction of HO-1 is an important protective response against the oxidative tissue injuries (Fig. **2**).

Recombinant human interleukin-11 (rhIL-11), a pleiotropic cytokine that induces megakaryocytic cell differentiation, is also known to exert a significant anti-inflammatory effect *via* its ability to suppress the production of pro-inflammatory mediators [28]. The reason why rhIL-11 is anti-inflammatory may be due to its ability to induce HO-1 in hepatocytes which had originally been shown in cultured human hepatoma cells [29]. Recently, it was also found that treatment of rats with rhIL-11 *in vivo* induced HO-1 both at transcriptional and protein levels in a highly liver-specific manner [30]. Following rhIL-11 treatment, the CCl_4-induced hepatic injury and inflammation were significantly suppressed, as judged by the reduction of elevated ALT activity, normalization of liver histology, and decreases in hepatic tumor necrosis factor (TNF) -α gene expression and DNA binding activity of nuclear factor (NF)-κB [30]. In contrast,

inhibition of HO activity by the administration of SnMP entirely abolished the beneficial effect of rhIL-11 also in this model [30]. HO-1 induction by rhIL-11 treatment thus plays an important role in the protection of the hepatic injury due to CCl_4-mediated oxidative damages, while inhibition of HO activity by SnMP significantly aggravates the tissue injury.

2.1.3. Chemopreventive/Chemoprotective Agents and HO-1 Induction

Some synthetic or natural compounds possess chemopreventive/chemoprotective as well as HO-1 inducing properties [31]. For instances, treatment of rats with 1,2-dithiole-3-thione, a cancer chemopreventive agent, resulted in a significant increase in hepatic heme oxygenase (HO) activity, as well as HO-1 protein [32]. Some organosulfar compounds such as diallyl sulfate, diallyl disulfate and diallyl trisulfide that are present in garlic also demonstrate pronounced chemopreventive effects [33]. Treatment of hepatoma HepG2 cells with various organosulfar compounds results in the activation of nuclear factor-E2-related factor (Nrf2), a redox-sensitive transcription factor, leading to the induction of HO-1 [34]. Isothiocyanate sulforaphane, a constituent of component of broccoli and some other cruciferous vegetables, has also been extensively investigated for its chemopreventive effects [31]. A recent study using HepG2 cells showed that sulphoraphane strongly induced Nrf2 protein expression and Maf recognition elements (MARE)-mediated transcription activation, retarded degradation of Nrf2 through inhibiting Keap1, and thereby activating the transcriptional expression of HO-1 [35]. The significant HO-1 inducing property of chemopreventive/chemoprotective substances suggests that they may represent an effective strategy to prevent liver from oxidative injuries.

2.2. HO-1 Induction is Necessary for the Reversal from Acute Renal Injury

Acute renal injury caused by various treatments, including ischemia-reperfusion, often appears to be due to a free heme-mediated oxidative tissue injury [5, 26, 36]. This question has been examined in several animal models that are summarized below.

2.2.1. Protection of Glycerol-Induced Acute Renal Injury by HO-1 Induction

The glycerol-induced acute renal injury is prepared by subcutaneous or intramuscular injection of hypertonic glycerol to rats, and is frequently used as an experimental model of acute renal injury [37]. This treatment causes a skeletal muscle injury, called "rhabdomyolysis", resulting in the rapid release of myoglobin into plasma, and HO-1 mRNA in the kidney increased more than 50-fold, compared with the untreated control [38]. The marked induction of HO-1 is presumably due to a large amount of myoglobin released into plasmas as a result of rhabdomyolysis (Fig. **2**). Thus, in contrast to the CCl_4-induced liver injury where free heme derived from hepatic CYP is the toxic principle, free heme derived from myoglobin appears to be directly responsible for attendant lipid peroxidation [39]. The inhibition of HO activity by tin protoporphyrin markedly aggravated the renal injury. In contrast, pre-induction of HO-1 by intravenous treatment of animals with hemoglobin led to significant improvement of the glycerol-induced renal injury [38]. Thus the preinduction of HO-1 by hemoglobin treatment facilitated the clearance of free heme, thereby protected the kidney from oxidative injury.

2.2.2. HO-1 Deficiency is Associated with Decreased Resistance Against Oxidative Damage

The role of HO-1 in acute renal injury caused by a heme/hemoprotein overload was also examined in HO-1 knockout mice [40]. Following the injection of hypertonic glycerol, wild-type mice which were able to induce HO-1 in response to the treatment sustained only mild renal insufficiency and not associated with any mortality [40]. In sharp contrast, homozygous HO-1 deficient mice developed severe renal failure and they all died within 15 days of glycerol injection [40]. The fundamental role of HO-1 expression was further supported by direct intravenous infusion of hemoglobin, which resulted in a mild sustainable kidney injury in wild type mice, whereas the same treatment in mutant HO-1 knockout mice led to severe acute renal failure and complete mortality [40]. A human patient with inherited HO-1 deficiency was also associated with extensive renal failure, and died at the age of 6 years of age [41]. These results thus have provided clear-cut evidence for the fundamental role of HO-1 induction in the protective response against acute renal injury.

2.2.3. Aggravation of Ischemic Renal Injury by HO Inhibition

Ischemic renal injury following reperfusion is a typical oxidative injury due to ROS generation [42], and is also known to accompany a rapid release of heme from microsomal CYP [43]. Renal HO-1 mRNA and its protein levels along with its enzyme activity were significantly increased within 30 min of reperfusion of rats that had been exposed to bilateral renal ischemia [44]. In uninephrectomized rats, 40 min of renal ischemia also significantly induced HO-1 mRNA and its enzyme activity after reperfusion of the kidney [45]. Inhibition of HO activity by SnMP resulted both in a rapid and marked increase in intracellular heme content, and in the aggravation of renal function [45]. Thus HO-1 induction in this model also clearly plays a critical role in the protection of the kidney from the heme-mediated oxidative damage.

2.2.4. Amelioration of Ischemic Renal Injury by Kidney-Specific HO-1 Induction

While tin is an essential trace element for some animals, it is not known whether it is essential to human health. While its deficiency is associated with growth retardation in rats [46], excessive concentrations of tin in man are known to cause various toxic effects such as gastrointestinal complaints [47]. A unique aspect of tin chloride is the fact that, while its intraperitoneal or intravenous administration to rats is highly toxic [48], its subcutaneous administration is non-toxic and is associated with marked induction of HO-1 in the kidney [49, 50]. Thus subcutaneous $SnCl_2$ pretreatment of rats significantly improved renal dysfunction due to ischemia/reperfusion, as judged by the suppression of increased

serum creatinine concentration [51]. There were also significant damages in proximal tubular cells in untreated control ischemic animals, whereas there was hardly any damage in the $SnCl_2$-pretreated animals [51]. Subcutaneous $SnCl_2$ treatment ensued in a marked elevation of renal HO-1 mRNA, followed by increases in HO-1 protein and its enzyme activity [51]. HO-1 protein was also shown to accumulate specifically in the renal tubular epithelial cells following $SnCl_2$ treatment. $SnCl_2$ treatment also significantly attenuated the sustained increase in microsomal heme concentration that was observed during ischemia/reperfusion [51]. In contrast, inhibition of HO activity by the administration of SnMP resulted in the accumulation of microsomal heme, and abolished the beneficial effect of $SnCl_2$ pretreatment on the ischemic renal injury [51], indicating the fundamental role of HO activity which removes the pro-oxidant "free heme", and protects renal epithelial cells from oxidative damages of ischemia/reperfusion.

2.3. Protective Role of HO-1 in the Septic Tissue Injury

Treatment of animals with lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, elicits systemic inflammation and symptoms similar to those observed in gram-negative bacterial sepsis [52]. LPS is responsible for the highly complex cascading events which lead to inflammatory tissue injuries [52]. In LPS-induced tissue injury, HO-1 was markedly induced [53], and ROS was also shown to be involved in this process [54] (Fig. **2**). Recently, Ets family transcription factors, which are known to play a vital role in the regulation of mammalian immunity [55], have been shown to be involved in the transcriptional regulation of *ho-1* gene by LPS [56, 57]. Namely, of several ternary complex factor subfamily members, Elk-3 has been shown to repress HO-promoter activity in macrophages [57]. Endotoxin treatment of these cells led to a dose-dependent decrease in endogenous Elk-3 mRNA levels and this reduction in Elk-3 preceded the LPS-mediated upregulation of HO-1 mRNA [57]. Binding of Elk-3 to the Ets-binding site-1 (EBS1) suppressed the transcriptional activity of *ho-1*, whereas LPS treatment decreased Elk-3 expression and its binding to EBS1, which thereby released cells from its basal state of *ho-1* repression [57].

2.3.1. The Intestinal Region with Higher HO-1 Expression is Better Protected than other Regions with Lower HO-1 Expression

The gastrointestinal tract plays a role in the pathogenesis of sepsis, both as a site of end-organ injury and as a contributor to immune activation *via* bacterial translocation [58]. In untreated control rats, the highest HO activity was found in the proximal small intestine, while the activities of the caecum and the large intestine were less than 30 % of that of the highest activity [59]. Similar to basal HO activity, HO-1 activity following intraperitoneal LPS treatment showed a marked increase in the mucosal epithelial cells in the upper intestine such as the duodenum and the jejunum, whereas it was hardly induced in the lower intestine such as the ileum and the colon [60]. In contrast, the intestinal tissue injury and inflammation were more pronounced in the lower intestine than in the upper intestine [60]. These findings suggest that the intestinal site where HO-1 can be induced during acute inflammatory stress is better protected from the oxidative tissue injuries than other sites where HO-1 cannot be induced. Consistent with this finding, pretreatment of animals with SnMP which augmented TNF-α gene expression resulted in mucosal epithelial cell injuries in the upper intestine, but not in the lower intestine [60]. Thus HO-1 induction and the maintenance of its activity are critical in the protection of the intestinal epithelial cells from oxidative injury induced by LPS treatment.

2.3.2. Coordinate Expression of HO-1 and HCP1 in the Duodenum

Most heme is known to be absorbed in the proximal intestine where HO-1 is highly expressed, with absorptive capacity decreasing distally [61]. Very recently, HCP1, heme carrier protein 1, was identified [62]. HCP1 is an iron-regulated intestinal heme transporter protein, and is highly expressed in mice in the upper intestinal cells such as the duodenum and is markedly induced by hypoxia at the transcriptional level, while it is not detected nor induced in the ileum [62]. Thus, it can be speculated that LPS treatment, which elicits a hypoxic stress, may lead to the production of free heme in the upper intestinal tract such as duodenum which then results in the induction of HO-1 as well as in the induction of HCP-1. Thus, the HO-1 inductive property of the upper intestine may serve both for the protection of cells from the oxidative injury, and for the facilitation of mucosal iron uptake.

2.3.3. Protection of the Septic Tissue Injury by Glutamine by its Intestine-Specific HO-1 Induction

Glutamine is now recognized as an essential nutrient during serious injury and illness [63]. Glutamine has been shown to be beneficial in the prevention of infectious morbidity and mortality in seriously ill patients [64], by its ability to maintain the integrity of intestinal mucosal epithelium [65]. Interestingly, it was found that rats treated with glutamine showed marked induction of HO-1 mRNA and protein highly specifically in the mucosal cells of the lower intestine, i.e., the ileum and the colon [66]. Following glutamine treatment, LPS-induced intestinal tissue injury in the ileum and the colon was also significantly suppressed, compared to glutamine-untreated controls [66]. Thus, by virtue of its ileum/colon-oriented HO-1 inducing activity, glutamine pretreatment significantly ameliorated LPS-induced mucosal injury, inflammation, and apoptotic cell death [66]. Importantly, glutamine treatment also markedly decreased LPS-induced mortality of animals. In contrast, additional treatment with SnMP entirely abolished the beneficial effect of glutamine treatment [66], indicating the protective effect of glutamine in LPS-treated animals is principally mediated by its ability to induce HO-1 in the lower intestine.

3. THE CRITICAL ROLE OF FREE HEME IN HO-1 INDUCTION

While heme is required as the prosthetic group for hemoproteins which are necessary for cellular viability, an excess amount of free heme is highly toxic to cells, due to its ability to produce oxygen radicals [1, 2, 4]. Free heme is also highly lipophilic and readily intercalates into the lipid bilayer of

adjacent cells, and results in oxidative damages of the cytoskelton [1, 2]. Exposure of endothelial cells to hemin, an oxidized form of heme that is available as a chemical, stimulates the expression of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin [67-69] (Fig. **2**), probably through heme-mediated generation of ROS, which underscores various types of inflammatory tissue damages. As free heme that is released from methemoglobin can catalyze the oxidation of low density lipoprotein which in turn induces lipid peroxide formation and results in endothelial cytolysis, free heme may also be involved in a variety of oxidative tissue injuries associated with intravascular hemolysis such as acute renal failure [70] (Fig. **2**). Hemin treatment of rats has also been shown to increase the DNA binding activity of NF-κB in the liver in a dose-dependent manner, and to lead to the activation of pro-inflammatory cascade [24]. In contrast, inhibition of HO activity by SnMP resulted in a marked increase in microsomal heme content and in the aggravation of the CCl_4–induced liver injury and inflammation [24], as well as in the aggravation of renal dysfunction and injury induced by renal ischemia/reperfusion [45]. Thus, an enhanced and sustained increase in intracellular free heme concentration may likely exacerbate the oxidative tissue injury, and rapid HO-1 induction plays a key role in removing free heme, the pro-oxidant, to prevent cells from further damages due to the heme-mediated oxidative injury.

3.1. The Role of Bach1 in ho-1 Gene Regulation

Recently, it was found that a transcription repressor Bach1 is a heme-responsive transcription factor which acts as a repressor for *ho-1* gene activation [71] (Fig. **2**). The repressor function of Bach1 is lost by its heme binding followed by its nuclear export, which in turn leads to transcriptional activation of the *ho-1* gene through MARE [72, 73]. Heme also induced nuclear translocation of Nrf2, a partner molecule for the Maf family [74], and promotes stabilization of Nrf2 [75]. Thus, when intracellular heme concentration is increased, Bach1 is displaced from the MARE sequences by heme binding, which then allows binding of Nrf2 and other small maf proteins, ultimately resulting in transcriptional activation of target genes that include *ho-1* (Fig. **2**), *thioredoxin*, and *keratinocyte growth factor* [71]. The MARE is also required for the hypoxic repression of the human *ho-1* gene expression [76].

An appropriate control of the Maf/NF-E2 system by the switching on-off of Bach1 and Nrf2 binding to the MARE sequence appears to be critical for normal cellular functions. For example, it is known that its dysregulation leads to tumorigenesis [77]. Therefore, various repressors and activators, such as Bach1 and Nrf2, respectively, may be strongly involved in the transcriptional control of the *ho-1* gene. The fact that the DNA binding activity of Bach1 is regulated by heme suggests that the stability of the gene expression program by Bach1 is directly influenced by intracellular heme concentrations. It should be noted that Bach1 knockout mice showed also significant *ho-1* gene activation, suggesting that low-level HO-1 expression in the wild type mice under normal physiological conditions is due to repression by Bach1, rather than a lack of activation [78]. Thus, the Bach1-*ho-1* system is the first example in higher eukaryotes that involves a direct regulation of a transcription factor for an enzyme gene by its substrate [78].

4. METABOLIC CONSEQUENCES OF HEME CATABOLISM

As discussed above, HO breaks down the pro-oxidant heme into three elements, i.e., iron, biliverdin IXα and CO, thus resulting in a decrease in the pro-oxidant stress. Iron, which is an oxidant, is directly sequestered and inactivated by co-induced ferritin [79]. HO-1 has also been reported to prevent cell death by exporting intracellular iron from cells both *in vivo* [80] and *in vitro* [81]. Recent evidence suggests that the two other heme metabolites, i.e., biliverdin IXα and CO, have significant anti-oxidant properties.

4.1. The Role of Bile Pigments in the Protective Response Against Oxidative Injury

Biliverdin IXα is rapidly converted by BVR to bilirubin IXα. Both biliverdin IXα and bilirubin IXα, as well as their glucuronides, are potent anti-oxidants [82]. It was also shown that higher baseline serum bilirubin levels in men were correlated with a lower incidence of myocardial infarction, which was thought to be mediated by the inhibitory effect of bilirubin on the oxidation of low-density lipoproteins [83]. Recently, redox cycling of biliverdin to bilirubin by BVR has been demonstrated, which suggests that it would further amplify their anti-oxidant effects [84]. Consistent with this observation, BVR silencing has been shown to sensitize cells to stress by sodium arsenite, a classical activator of the HO-1 response [85].

BVR undergoes auto-phosphorylation in order to convert biliverdin to bilirubin [86]. This property of phosphorylation/dephosphorylation during the conversion of biliverdin to bilirubin is similar to that seen with other signaling kinases. Recent evidence also indicates that BVR functions not only as a serine/threonine kinase that operates in the insulin receptor/MAPK pathways [87], but also as a novel transcription factor with a bZip domain that regulates ATF-2/CREB and HO-1 expression [88]. These additional roles of BVR suggest that BVR may have a broader function in regulating cellular activity than it is used to be thought [89].

4.2. The Protective Role of CO Against Oxidative Injury

Recently, CO has also been shown to exhibit anti-inflammatory and anti-apoptotic properties, which are thought to be mediated, at least in part, by activation of the p38 MAPK signaling pathway [90, 91]. The anti-inflammatory effect of CO also appears to involve the JNK pathway and AP-1 [92]. In addition, it has also been reported that the anti-apoptotic effect of CO involves inhibition of Fas/FasL expression, and other apoptosis-related factors including caspases, mitochondrial cytochrome-c release, Bcl-2 proteins and poly (ADP-ribose) polymerase cleavage [93]. Heat shock protein 70 has also been implicated in mediating the cytoprotective effect of CO [94]. Thus, in addition to the removal of the pro-oxidant heme by HO, and the generation of anti-oxidants biliverdin/bilirubin, the HO-mediated CO production may also play a role, in concert with other heme metabolites, in the protective response against oxidative stimuli, and contribute to the suppression of oxidative tissue injuries.

4.2.1. Increased CO Concentration in Exhaled Air in Patients with Respiratory Inflammation

The majority of CO formed in the body is derived from heme degradation [95, 96], and 80% of it is excreted into the exhaled air [97]. Thus, measurement of CO in exhaled air may potentially be useful in monitoring changes in HO enzyme activity *in vivo*, which might reflect the degree of inflammation or oxidative stress in patients (Fig. **2**). In fact, several groups reported that there are increased CO concentrations in exhaled air in patients with inflammatory respiratory disease such as asthma, upper respiratory tract infection, and cystic fibrosis [98-102], and these levels appeared to be influenced by therapy or exacerbations [99, 102-104]. These findings were interpreted to suggest that inflammatory reaction in the respiratory tract may have been responsible for HO-1 induction, thereby for increases in exhaled CO concentration.

4.2.2. Increased CO Concentration in Exhaled Air in Patients with Systemic Inflammation

Exhaled CO concentrations as well as arterial CO-Hb concentrations in patients with non-respiratory disorders were also examined and found significantly increased after surgery. There were no differences in these indices between spinal anesthesia and general anesthesia [105]. In these patients, CO generated by HO reaction is thought to diffuse out of cells, enter the blood to form CO-Hb and is transported to the lungs where it is excreted into ambient air [106]. Thus, in addition to patients with respiratory inflammations, patients with systemic non-respiratory inflammation may also be associated with an increase in CO concentration in the exhaled air. Cumulative evidence also suggests that exhaled CO level is increased in critically patients with systemic inflammation of various nature.

Total CO production, which can be calculated by CO concentration in exhaled air and respiratory minute volume, was also increased in critically ill patients, suggesting that these patients may have increased HO-1 activity [107]. When the endogenous CO production was specifically calculated as the lung CO excretion rate at a steady state, a significantly higher endogenous CO production was found in patients with severe sepsis compared to critically ill controls [108]. Exhaled CO concentrations were significantly increased in critically ill patients compared with those in healthy volunteers, and were also significantly correlated to arterial CO-Hb concentrations and serum total bilirubin IXα concentrations [109] These findings suggest that there may be an increase in heme breakdown in critically ill patients [109]. The mechanism behind the increased heme breakdown in these patients has not been fully elucidated. However, metabolic stress such as fasting and hypoglycemia that occurs in critically ill patients is known to increase heme catabolism [110]. Thus, in addition to oxidative stress due to systemic inflammation, catabolic conditions in these patients may also contribute to the increased heme breakdown. When exhaled CO concentrations in 95 mechanically ventilated critically ill patients were measured, endogenous CO production was found to correlate with the severity of acute illness and with the multiple organ dysfunction score [111]. Moreover, patients suffering from cardiac diseases as well as critically ill patients undergoing dialysis were found to produce significantly higher amounts of CO compared to critically ill controls [111]. These findings thus appear to suggest that endogenous CO production may reflect the severity of acute organ dysfunction. Further studies are clearly needed as the correlation among these indices is yet weak to moderate, and it remains unclear whether increased endogenous CO production may predict the patient's morbidity and mortality. Techniques for monitoring CO are, however, continuously being refined and this technique may eventually find their way into the office of clinicians.

CONCLUSIONS

In this review, we summarized recent evidence concerning the role of free heme in the oxidative tissue injury, and HO-1 induction as a major protective response against the free heme-mediated oxidative toxicity observed in various acute inflammatory disorders. Preinduction of HO-1 by pharmacological means has been shown to confer significant protection on cells, tissues and organs in various experimental models of acute or systemic inflammatory disorders. We also described a novel non-invasive technology for the measurement of exhaled CO concentrations which reflect endogenous HO activity and might be a useful parameter of disease severity. In addition to the protective role of HO-1, both bile pigments and CO, the two heme metabolites by HO reaction, play pivotal tissue-protective roles in the anti-oxidative tissue injuries. Further studies should clarify pending issues such as interspecies, or inter-cell type differences in *ho-1* gene expression [112], and a cause-effect relationship between HO-1 expression and morbidity and mortality of patients, which are necessary for a better prediction from preclinical to clinical studies.

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ABBREVIATIONS

ALT = Alanine aminotransferase

BVR = Biliverdin reductase

CCl_4 = Carbon tetrachloride

CO = Carbon monoxide

CO-Hb = Carboxyhemoglobin

CR = NADPH-cytochrome P450 reductase

CYP = Cytochrome P450

EBS1 = Ets-binding site-1

HO = Heme oxygenase

LPS = Lipopolysaccharide

MARE = Maf recognition element

NF = Nuclear factor

Nrf2 = Nuclear factor-E2-related factor

ROS = Reactive oxygen species

$SnCl_2$ = Tin chloride

SnMP = Tin mesoporphyrin

TNF = Tumor necrosis factor

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