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Heme Proteins, Heme Oxygenase-1 and Oxidative Stress

Hiroshi Morimatsu, Toru Takahashi, Hiroko Shimizu, Junya Matsumi, Junko Kosaka and Kiyoshi Morita
Department of Anesthesiology and Resuscitology, Okayama University Hospital, Japan

1. Introduction

Heme is ferrous protoporphyrin-IX that is the prosthetic group of hemoproteins, such as hemoglobin, myoglobin and cytochromes that are of vital importance. In contrast, "free heme", a protein-unbound heme, that is either just synthesized but yet not incorporated into hemoproteins, or that is released from hemoprotein under oxidative conditions, is highly toxic, since it catalyzes the production of reactive oxygen species (ROS). Thus, heme proteins and free heme have an important relationship with oxidative stress.

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. The rate-limiting enzyme in heme catabolism, heme oxygenase-1 (HO-1), is induced by not only its substrate heme but also oxidative stress resulting from I/R injury. Heme oxygenase-1 induction leads to increased heme breakdown, resulting in the production of iron, carbon monoxide (CO), and biliverdin IXα, which is subsequently reduced to bilirubin IXα by biliverdin reductase.

Recently, large numbers of reports including ours have emerged suggesting heme proteins, HO, and its substrates such as CO, biliverdin IXα, and bilirubin IXα play important roles in pathophysiology and therapeutic implications. Here we summurize these evidences to clarify the relationship among heme proteins, HO-1, and oxidative stress.

1.1 Synthesis and degradation of heme protein 1

Heme is the prosthetic group of all heme proteins such as hemoglobin, myoglobin, cytochrome, catalase, peroxidases, nitric oxide synthase, prostaglandin synthase, and certain transcription factors. Heme is an essential molecule in all aerobic cells and plays a crucial role in physiological, pharmacological, and toxicological reactions, as well as cell differentiation and other functions. However, free heme, namely protein-unbound heme, can be toxic to cells because it results in the production of reactive oxygen species and

causes cell damage (Kumar and Bandyopadhyay, 2005). To guard against this toxicity, heme levels are tightly controlled between heme biosynthesis and catabolism (Sassa, 2006).

1.1.1 Heme synthesis

The initial biosynthesis of one molecule of heme requires eight molecules of glycine and eight molecules of succinyl CoA to produce 5-aminolevulinic acid (ALA) (Sun et al., 2002) by 5-aminolevulinic acid synthase (ALAS) in mitochondria. There are two forms of ALAS, a non-tissue-specific ALAS (ALAS1) and an erythroid cell-specific ALAS (ALAS2) (Bishop et al., 1990). In the liver, heme represses the synthesis of ALAS1 mRNA at both transcriptional and translational levels (Hamilton et al., 1991) and inhibits its transfer from the cytosol into mitochondria (Ades and Harpe, 1981). In erythroid cells, heme does not inhibit ALAS2 synthesis (Sassa and Nagai, 1996) and ALAS2 activity (Ponka, 1997).

Following synthesis, mitochondrial ALA is transported to the cytosol, where ALA dehydratase (ALAD) dimerizes two molecules of ALA to produce the pyrrole ring compound porphobilinogen (PBG). The next step in the pathway involves the head-to-tail condensation of four moleclues of PBG to produce the linear tetrapyrrole intermediate hydroxymethylbilane (HMB). The enzyme for this condensation is porphobilinogen deaminase (PBG deaminase), also called hydroxymethylbilane synthase or uroporphyrinogen I synthase. Uroporphyrinogen-III synthase catalyses HMB to uroporphyrinogen III. In the absence of uroporphyrinogen-III synthase, HMB may non-enzymatically close to form uroporphyrinogen I, which cannot convert to heme.

In the next step, the acetate substituents of uroporphyrinogen III or I are all decarboxylated by the uroporphyrinogen decarboxylase in the cytosol. The resultant products are known as coproporphyrinogens, with coproporphyrinogen III being the important normal intermediate in heme synthesis.

Coproporphophyrinogen III is transported into mitochondria and is catalyzed to protoporphyrinogen IX by coproporphyrinogen oxidase. Protoporphyrinogen oxidase oxidizes protoporphyrinogen IX to protoporphyrin IX by the removal of six hydrogen atoms. Finally, ferrous iron (Fe^{2+}) is inserted into protoporphyrin IX to form heme in a reaction catalysed by ferrochelatase.

1.1.2 Heme degradation

Heme degradation starts with the reductive breakdown of the heme into carbon monoxide (CO), iron (Fe), and biliverdin in a reaction catalyzed by heme oxygenase (HO) (Tenhunen et al., 1968). Heme oxygenase exists in two isoforms, HO-1, which is inducible by heme, its substrate, and HO-2, which is constitutive and non-inducible (Shibahara et al., 1985). Heme oxygenase-1 is also known as heat shock protein 32 (Keyse and Tyrrell, 1989), as well as an acute phase reactant, and it is inducible by stressors including cytokines, heavy metals, hypoxia, and oxygen free radicals. This is the only reaction in the body that is known to produce CO. Most of the CO is excreted through the lungs, so that the CO content of expired air is a direct measure of the activity of heme oxygenase. Biliverdin is subsequently converted into bilirubin by an NAD(P)H-requiring cytosolic enzyme, biliverdin reductase

(Tenhunen et al., 1969). Bilirubin is conjugated with glucronic acid to form a more soluble bilirubin glucuronide, which is excreted in bile.

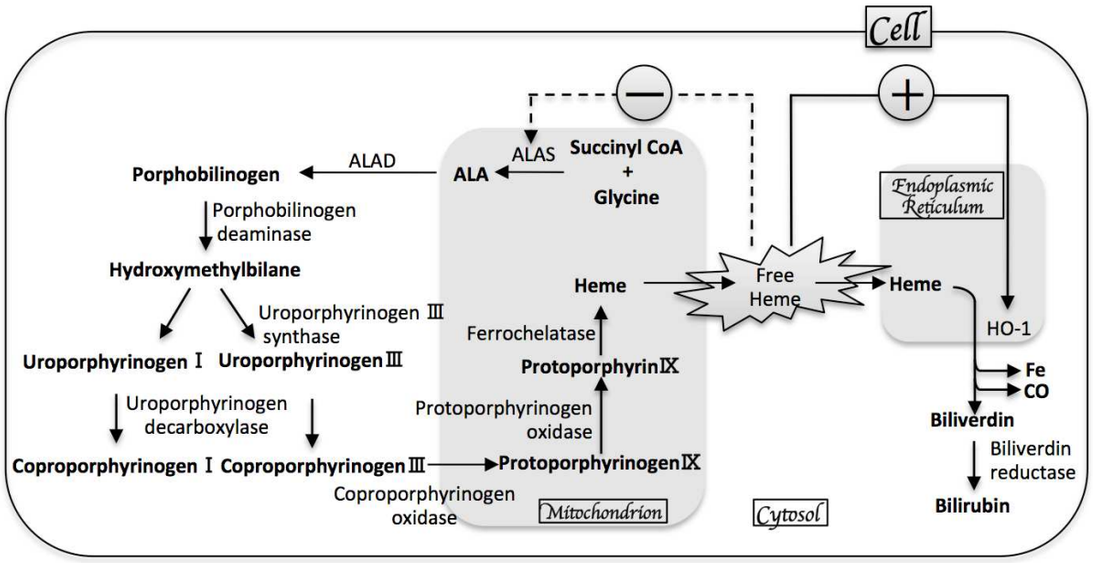

Fig. 1. Heme Metabolic Pathway

1.1.3 The regulatory effects of free heme

Free heme at low concentrations plays a beneficial regulatory role on various cellular functions. A heme concentration greater than 1 μM can be toxic to almost all cells because it catalyzes the production of reactive oxygen species (Halliwell and Gutteridge, 1990). At submicrosomal concentrations, heme is involved in regulator gene expression or repression of heme metabolism.

Heme concentrations of less than 10^{-13} M induce the synthesis of ALAS1. Repression of ALAS1 synthesis in the liver takes place at free heme concentrations of 0.1-0.3 μM, leading to decreased heme synthesis, and at 0.4-1.0 μM, HO-1 is induced in cultured chick embryo liver cells (Granick et al., 1975). In 1996, Igarashi reported two novel transcription factors, Bach1 and Bach2, as heterodimerization partners of MafK (Oyake et al., 1996). In the early 2000s, it was reported that the mammalian transcription factor Bach1, a repressor of HO-1 gene activation (Sun et al., 2002), binds with an equimolar amount of hemin (Ogawa et al., 2001). Inhibition by free heme of the DNA binding activity of Bach1 occurred at around 0.03 μM, and at 1 μM, it almost completely inhibited the DNA-binding activity of Bach1 in vitro (Ogawa et al., 2001). In heme oxygenase deficiency, hemin applied at 50 μM to the patient's plasma resulted in increased free radical generation, which was abnormal and caused varied tissue damage (Poss and Tonegawa, 1997). The products of heme degradation, CO, iron Fe, and biliverdin, contribute to cellular protection in various situations (Sassa, 2006). Bilirubin is considered a potentially important anti-oxidant and cytoprotector of physiological significance (Stocker et al., 1987) (Gopinathan et al., 1994) (Hopkins et al., 1996). Thus, heme levels are tightly controlled between heme biosynthesis and catabolism.

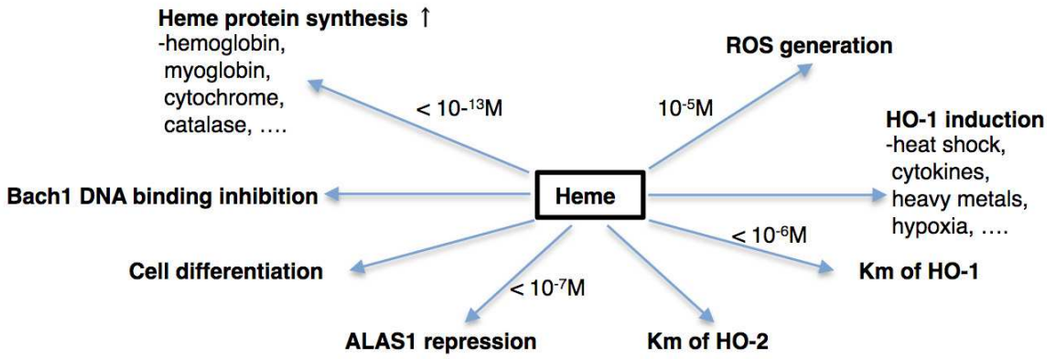

Fig. 2. The regulatory effects of free heme

2. Heme proteins as oxidants

While heme is required as the prosthetic group for heme proteins such as hemoglobin, myoglobin, and cytochrome P 450, etc., which are necessary for cellular viability, an excess amount of free heme is highly toxic to cells due to its pro-oxidant activity, driven by the divalent Fe atom contained within its protoporphyrin IX ring, which can promote the production of free radicals via Fenton chemistry (Sassa, 2006). Free heme is also highly lipophilic and readily intercalates into the lipid bilayer of adjacent cells, and it results in oxidative damage of the cytoskeleton. Furthermore, 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 (Jeney et al., 2002).

2.1 Exacerbation of oxidative tissue injury by free heme

We have demonstrated that free heme released from heme protein plays a critical role in the development of oxidative tissue injuries by accelerating the production of reactive oxygen species (ROS) in various experimental models of oxidative tissue injuries (Takahashi et al., 2007). For instance, ROS generated by reperfusion of the kidney has been implicated in the pathogenesis of ischemic renal injury. Thus, we determined the level of microsomal heme and the gene expression of ALAS1 in the kidney following ischemia/reperfusion in rats (Shimizu et al., 2000). We found that, prior to HO-1 induction, there was a rapid and significant increase in microsomal heme concentration, which was followed by the inhibition of ALAS1 gene expression. These findings indicate that free heme concentration in the kidney increases rapidly following ischemia/reperfusion. We also found that inhibition of HO activity by tin-mesoporphyrin, a specific competitive inhibitor of HO activity, resulted in a marked increase in microsomal heme content and in the aggravation of ischemic renal injury (Shimizu et al., 2000). Thus, an enhanced and sustained increase in intracellular free heme concentration derived from cytochrome P450, a major heme protein in the kidney, may likely exacerbate the oxidative tissue injury in the kidney caused by renal ischemia/reperfusion.

2.1.2 Activation of the innate immune response by free heme

Recent studies also indicate that free heme is involved in the activation of innate immunity, which can lead to oxidative tissue injury. 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 (Wagner et al., 1997). Hemin also induces neutrophil migration *in vivo* and *in vitro*, triggers the oxidative burst, promotes cytoskeleton reorganization, and activates interleukin-8 expression in human neutrophils (Graca-Souza et al., 2002). Heme also induce TNF-α secretion by mouse peritoneal macrophages in a manner dependent on MyD88, toll-like receptor (TLR) 4, and CD14, although heme signaling through TLR4 depends on an interaction distinct from that established between TLR4 and LPS (Figueiredo et al., 2007). Moreover, free heme induces apoptotic cell death in response to pro-inflammatory agonists, as demonstrated for tumor necrosis factor (Seixas et al., 2009). Severe sepsis can develop from excessive systemic inflammatory responses to microbial infection, leading to oxidative tissue injury that ultimately results in death. Very recently, the circulating free heme released from hemoglobin during infection has been shown to contribute to the pathogenesis of severe sepsis (Larsen et al., 2010). Heme administration after low-grade polymicrobial infection induced by cecal ligation and puncture in mice promoted tissue damage and severe sepsis. Development of lethal forms of severe sepsis after high-grade infection was associated with the increase in plasma free heme concentration derived from cell-free hemoglobin and the decrease in serum concentrations of the heme sequestering protein hemopexin (HPX), whereas HPX administration after high-grade infection prevented tissue damage and lethal outcomes. Moreover, fatal septic shock in patients was associated with reduced serum HPX concentrations, suggesting that targeting free heme by HPX might be used therapeutically to prevent lethal outcomes associated with severe sepsis.

2.2 Role of HO-1 in oxidative tissue injury (liver disease and sepsis)

Oxidative stresses such as inflammation, as well as ischemia and reperfusion (I/R), injure several tissues. It has been suggested that HO-1 plays a cytoprotective role against oxidative stresses. The cytoprotective role of HO-1 influences both acute and chronic illnesses. In this chapter, we evaluate the role of HO-1 in protection against oxidative stresses at acute illnesses, mainly liver disease and sepsis.

2.2.1 Animal studies

In animal models, several reports have demonstrated the protective effect of HO-1. In the carbon tetrachloride-induced hepatotoxicity model, HO-1 expression is increased both at transcriptional and protein levels in hepatocytes. Inhibition of HO activity by tin-mesoporphyrin (Sn-MP) results in sustained liver injury, as revealed by marked increases in serum alanine transaminase (ALT), hepatic malondialdehyde formation, tumor necrosis factor-alpha (TNF-α) mRNA, inducible nitric oxide synthase (iNOS) mRNA, and DNA-binding activity of nuclear factor-kappaB (NF-κB), as well as inflammatory changes of hepatocytes (Nakahira et al., 2003). In contrast, induction of HO-1 by recombinant human interleukin-11 (rhIL-11) leads to reduced liver injury. (Kawakami et al., 2006) In the I/R liver injury model, rats pretreated with a HO-1 inducer showed greater increases in HO-1 transcriptional and protein expressions, less elevated serum ALT levels, and less increased serum TNF-α and iNOS protein and mRNA expressions than those treated with a HO-1 inhibitor. These results indicated that HO-1 overexpression protected liver against I/R injury by modulating oxidative stress and proinflammatory mediators (Yun et al., 2010). In

sepsis models, lipopolysacchalide (LPS) treatment increases HO-1 at transcriptional and protein levels and decreases nonspecific delta-aminolevulinate synthase (ALAS-1), which are the rate-limiting enzymes of heme catabolism and biosynthesis, gene expression in the duodenum and the jejunum. Inhibition of HO activity by Sn-MP produces significant tissue injury (Fujii et al., 2003). LPS also induces hepatic injury as revealed by increases in serum ALT and aspartate transaminase (AST) activities, TNF-α mRNA, iNOS mRNA, and DNA-binding activity of NF-κB, and extensive hepatocyte necrosis. However, induction of HO-1 by rhIL-11 ameliorated the LPS-induced hepatic injury and decreased LPS-induced mortality (Maeshima et al., 2004) In an animal model, the cytoprotective effects of HO-1 against oxidative stress were also shown in other organs (Maeshima et al., 2005, Barreiro et al., 2002, Shimizu et al., 2000, Poole et al., 2005, Yu et al., 2009).

2.2.2 Human studies

In humans, there are some reports indicating the protective effect of HO-1. Patients with acute liver failure show increased HO-1 and decreased ALAS-1. These may indicate an increase in free heme concentration, resulting in altered heme metabolism and liver function (Fujii et al., 2004). In liver transplantation, which induces oxidative stress through I/R, a donor HO-1 genotype that modulates HO-1 induction levels is associated with outcomes, such as serum ALT and AST levels and early graft survival. This result suggests that HO-1 mediates graft survival after liver transplantation (Buis et al., 2002). In sepsis and septic shock, patients who fulfilled the criteria for severe sepsis or septic shock showed high HO-1 gene expression, and there was a positive correlation between survival and increased HO-1 concentration (Takaki et al., 2010). Patients who fulfilled the criteria for severe systemic inflammatory response syndrome and had a serum C-reactive protein level >10 mg/dL showed high HO-1 expression and serum TNF-α levels. (Mohri et al., 2006) These results indicate the relationship between inflammation and HO-1. A patient with HO-1 deficiency showed growth retardation, anemia, leukocytosis, thrombocytosis, coagulation abnormalities, elevated serum levels of haptoglobin, ferritin, and heme, a low serum bilirubin concentration, and hyperlipidemia; the patient died in childhood (Kawashima et al., 2002) This case directly shows the importance of HO-1 in homeostasis.

In summary, similar results in animal models and humans have shown the cytoprotective effect of HO-1 against oxidative stresses. The complete mechanisms related to the cytoprotective effect of HO-1 against oxidative stresses are still unknown, but several mechanisms may be involved. The major mechanism may be the removal of free heme. In oxidative stress, free heme is increased with the breakdown of hemoproteins such as hemoglobin, myoglobin, or cytochrome P450. Free heme induces the production of reactive oxygen species and low-density lipoprotein oxidation, which injures endothelial cells (Sassa, 2006). Another major mechanism may be the anti-oxidative effect of carbon monoxide and biliverdin, which are produced in heme catabolism. The detailed mechanisms related to the anti-oxidative effects of carbon monoxide and biliverdin are described in other chapters. One of the other possible mechanisms is the decrease of cytotoxic cytokines. HO-1 may affect many pathways and cytokines. For example, HO-1 inhibits macrophage activation, which triggers the inflammatory response in response to stress. In the liver, Kupffer cells, which are liver macrophages, play an important role for these reactions, such as production of TNF-α and IL-6. HO-1 inhibits the production of these cytokines by Kupffer cells and

ameliorates liver damage (Babu et al., 2007, Zhong et al., 2010, Devey et al., 2009). In addition to macrophage activation, there may also be many other mechanisms, such as inactivation of the p38 mitogen-activated protein kinase pathway, which leads to a preventive effect by diminishing neutrophil infiltration. (Carchman et al., 2011, Lin et al., 2010).

In conclusion, even though the detailed mechanisms are unknown, HO-1 is one of the essential enzymes acting against oxidative stress, and its cytoprotective effect operates in many organs and probably affects patients' outcomes. More investigations into the detailed role and mechanisms of HO-1 are needed.

2.3 Bilirubin as an antioxidant

Bilirubin has been recognized as a marker of liver injury, specifically biliary obstruction. It is also well known that biliverdin is one of the metabolites catalyzed by heme oxygenase from heme proteins, and it is catalyzed by biliverdin reductase to bilirubin. An increased serum bilirubin concentration is seen as a sign of dysfunction in the hepato-billiary system or in heme protein metabolism. Free unconjugated bilirubin (UCB) can easily enter cells by passive diffusion and cause toxicity. UCB binds to discrete brain areas, such as the basal ganglia (kernicterus), and produces a wide array of neurological deficits collectively known as bilirubin encephalopathy (Shapiro, 2003). However, in 1987, it was noted that bilirubin has strong antioxidant potential *in vitro* (Stocker et al.,1987). In this study, bilirubin under 2% oxygen in liposomes had a stronger antioxidant potential than α-tocopherol known to date as the most potent protector against lipid peroxidation. This result showed that endogenous bile pigment production activated by elevated HO activity could confer antioxidative protection to cells and tissues. In another study, the potent physiologic antioxidant actions of bilirubin were reported to involve a redox cycle between bilirubin and biliverdin (Baranano et al., 2002). When bilirubin acted as an antioxidant, it was itself oxidized to biliverdin and then recycled by biliverdin reductase back to bilirubin.

2.3.1 The antioxidant and cytoprotective effects of bilirubin in animal studies

In several animal models, the antioxidant potential and cytoprotective effect of bilirubin were also reported. In an I/R heart injury model, HO-1 and bilirubin showed a protective effect with respect to postischemic myocardial performance and reduced infarct size and mitochondrial dysfunction (Clark et al., 2000). In experimental small intestinal I/R injury, bilirubin had a dose-dependent protective effect by preventing lipid peroxidation (Ceran et al., 2001). In this study, bilirubin infusion reduced the severity of postischemic intestinal injury and increased tissue malondialdehyde (MDA) levels. Malondialdehyde is a product of lipid peroxidation. Moreover, exogenous bilirubin infusion provided tissue protection in other models of hepatic (Kato et al., 2003) and renal (Adin et al., 2005) I/R injury. In an OVA-induced asthma model, the application of bilirubin inhibited airway inflammation and lung leukocyte influx (Keshavan et al., 2005). Bilirubin also inhibited vascular cell adhesion molecule 1 (VCAM-1)-mediated transendothelial lymphocyte migration *in vitro*. The authors suggested that bilirubin inhibited the cellular production of ROS in responce to VCAM-1 stimulation as an antioxidant. Furthermore, rats rendered hyperbilirubinemic by infusion of bilirubin were relatively resistant to bleomycin-induced lung injury (Wang et al., 2002). Intravenous infusion of bilirubin reduced lung fibrotic lesions and local infiltrations of

inflammatory cells in histologic studies, as well as reduced levels of transforming growth factor-β (TGF-β) in the bronchoalveolar lavage fluid.

2.3.2 The relationship between serum bilirubin levels and the risk of general diseases

In several studies, mild to moderately elevated serum bilirubin levels were effective in the prevention of general diseases related to oxidative stress in humans (Ryter et al., 2007). For example, some clinical studies have indicated correlations between the serum bilirubin level and the risk of cardiovascular disease. For coronary artery disease (CAD), the relationship between serum bilirubin levels and the risk was investigated (Schwertner et al., 1994). In their study, the total bilirubin level was inversely related to the incidence of CAD independently. In the Framingham offspring study (large scale cohort study, n=5124), the relationship between serum bilirubin and myocardial infarction, coronary death, and any cardiovascular event was assessed (Djousse et al., 2001). Participants were divided into five groups by serum bilirubin level and compared. It was found that higher serum total bilirubin levels were associated with a lower risk of cardiovascular disease in men. Moreover, middle-aged patients with Gilbert syndrome (with serum bilirubin levels in the range of 20-70 μmol/l) had a lower incidence of ischemic heart disease (IHD) than healthy patients (Vitek et al., 2002). In this study, the authors referred to the total antioxidant potential of UCB. They concluded that the beneficial effect of UCB on the prevention of IHD might be important, in addition to HDL cholesterol.

The serum bilirubin level was shown to be associated with respiratory disease (Temme et al., 2001, Horsfall et al., 2011). In two studies, the relationship between serum bilirubin level and respiratory disease was examined. They reported that the serum bilirubin level was inversely correlated with the incidence of respiratory disease (lung cancer, chronic obstructive pulmonary disease) and all-cause mortality.

In conclusion, bilirubin has a strong antioxidant potential and cytoprotective effect *in vitro* and *in vivo*. The antioxidant potential of bilirubin involves a redox cycle between bilirubin and biliverdin. An elevated serum bilirubin level is associated with the incidence and the mortality of several diseases induced by oxidative stress. However, hyperbilirubinemia causes brain damage in infants and neonates. Thus, further investigations of the antioxidative and cytoprotective mechanisms of bilirubin are needed.

2.4 Carbon monoxide as an indicator of oxidative stress

Carbon monoxide (CO) is also one of the metabolites of heme proteins. It is well known that CO is a toxic gas and is used as an indicator of air pollution. Recent studies suggest that CO inhalation in very low concentration would be a therapeutic option in experimental models of sepsis, transplantation, and ischemia/reperfusion. Currently, CO concentration can be measured using two methods: CO-hemoglobin using a blood gas analyzer, and exhaled CO using a gas sampler. These new measurements will provide us important new information about patient status and underlying mechanisms of disease.

2.4.1 Increased CO concentration in exhaled air of critically ill patients

Zegdi et al. (2000) focused their attention on the exhaled CO concentration of critically ill patients, and they measured CO concentrations using an infrared CO analyzer with a

sensitivity of 0.1 ppm (CO 2000, Seres, La Duranne, France). Carbon monoxide was detected in exhaled breath at a higher concentration than in inspired gas, and exhaled CO was constant at the fixed ventilator settings in hemodynamically stable patients. They suggested that the exhaled CO concentration reflects endogenous CO production and might be useful for assessing the condition of critically ill patients. Coincident with their report, Sharte and colleagues measured exhaled CO concentrations in 30 critically ill patients who underwent mechanical ventilation and compared their results to those of 6 healthy non-smoking controls without a recent history of respiratory infections who breathed spontaneously via a mouthpiece connected to a ventilator (Sharte et al., 2000). Critically ill patients showed significantly higher CO concentrations in exhaled air compared to healthy controls. Although they did not find correlations between CO concentrations in exhaled air and carboxyhemoglobin levels in arterial and central venous blood, this might be attributable to technical artifacts in the measurement of carboxyhemoglobin concentrations using an older version of the blood gas analyzer, which has a lower sensitivity. Taken together, they concluded that the increased CO concentration in exhaled air in critically ill patients suggests an induction of inducible HO-1 and might reflect the severity of illness. Since CO is one of the metabolites of heme catabolism, we also examined CO concentrations in exhaled air, carboxyhemoglobin concentrations in arterial blood, and serum levels of bilirubin, another metabolite of heme breakdown, in 29 critically ill patients with signs of systemic inflammation who were all being mechanically ventilated (Morimatsu et al., 2006). Exhaled CO concentrations were also measured in eight healthy volunteers as controls. Exhaled CO concentration was measured using the CO analyzer (CARBOLYZER mBA-2000; TAIYO Instruments, Osaka, Japan). The median exhaled CO concentration was significantly higher in critically ill patients than in controls. Of note, there was a significant correlation between CO and carboxyhemblobin, and between CO and total bilirubin levels. We also compared exhaled CO concentrations between survivors and nonsurvivors. Interestingly, survivors tended to have higher exhaled CO concentrations than nonsurvivors, but the difference was not significant because of the limited sample size, suggesting that the poorer outcome of nonsurvivors may be due to their limited capacity to produce CO or induce HO-1. Collectively, our findings suggest that there may be an increase in heme breakdown in critically ill patients, probably due to systemic oxidative stress.

2.4.2 Increased CO concentration in exhaled air in patients with systemic inflammation/sepsis

Schober et al. (2009) measured end-tidal CO concentrations and arterial CO-Hb concentrations in 20 patients undergoing cardiac surgery with cardiopulmonary bypass (CPB). They measured these indices during surgery at two time points (1 hour after induction and 1 hour after CPB). They compared pre- and post-CPB values and found that both the end-tidal CO and the arterial CO-Hb concentrations were higher post-CPB than pre-CPB. These results indicated that systemic inflammation induced by CPB resulted in oxidative stress and increased CO production. This is likely explained by specific influences of CPB on processes involved in heme degradation, such as HO-1 induction and/or hemolysis. In addition, Zegdi et al. (2002) measured the exhaled CO concentrations in 24 patients with severe sepsis or septic shock who were admitted to a medical intensive care unit and compared them to those of 5 critically ill controls. All patients were mechanically ventilated. They demonstrated for the first time that exhaled CO concentrations were

greater in the septic patients than in the control group. When endogenous CO production was specifically calculated as the lung CO excretion rate at a steady state in these patients, significantly higher endogenous CO production was found in patients with severe sepsis during the first three days of treatment than in the control group, although endogenous CO production in the sepsis group decreased over time with treatment. Interestingly, survivors of sepsis had a significantly higher endogenous CO production on day 1 compared to non-survivors.

We summarized recent evidence concerning the increased exhaled CO concentrations and its significance in critically ill patients with systemic inflammation. The exhaled CO concentration could reflect endogenous HO activity and might be a useful parameter of oxidative stress. Further studies are clearly needed to elucidate whether increased endogenous CO production may predict patients' morbidity and mortality. However, techniques for monitoring CO are continuously being refined, and these techniques may eventually find their way into clinicians' offices.

3. Conclusion

In this chapeter, we showed 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 tissue injuries, especially focusing on acute liver injuries and septic organ damages. Preinduction of HO-1 by pharmacological modality has been shown to confer significant protection on cells, tissues and organs in these acute 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 critical tissue-protective roles agaisnt oxidative tissue injuries. Although the application of HO-1 and its metabolites to clinical field might be promising, further studies should clarify pending issues such as interspecies, or inter-cell type differences in ho-1 gene expression, and a cause-effect relationship between HO-1 expression and morbidity and mortality of patients.

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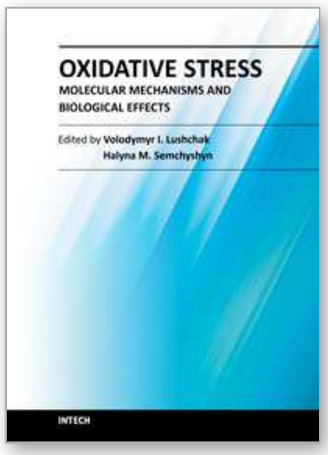

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Since the discovery of free radicals in biological systems researchers have been highly interested in their interaction with biological molecules. Denoted in 1980, and due to fruitful results and ideas, oxidative stress is now appreciated by both basic and applied scientists as an enhanced steady state level of reactive oxygen species with wide range of biological effects. This book covers a wide range of aspects and issues related to the field of oxidative stress. The association between generation and elimination of reactive species and effects of oxidative stress are also addressed, as well as summaries of recent works on the signaling role of reactive species in eukaryotic organisms. The readers will gain an overview of our current understanding of homeostasis of reactive species and cellular processes they are involved in, as well as useful resources for further reading.

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