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Heme Oxygenase-1: A Metabolic Nike

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Abstract

Significance: Heme degradation, which was described more than 30 years ago, is still very actively explored with many novel discoveries on its role in various disease models every year. **Recent Advances:** The heme oxygenases (HO) are metabolic enzymes that utilize NADPH and oxygen to break apart the heme moiety liberating biliverdin (BV), carbon monoxide (CO), and iron. Heme that is derived from hemoproteins can be toxic to the cells and if not removed immediately, it causes cell apoptosis and local inflammation. Elimination of heme from the milieu enables generation of three products that influences numerous metabolic changes in the cell. **Critical Issues:** CO has profound effects on mitochondria and cellular respiration and other hemoproteins to which it can bind and affect their function, while BV and bilirubin (BR), the substrate and product of BV, reductase, respectively, are potent antioxidants. Sequestration of iron into ferritin and its recycling in the tissues is a part of the homeodynamic processes that control oxidation-reduction in cellular metabolism. Further, heme is an important component of a number of metabolic enzymes, and, therefore, HO-1 plays an important role in the modulation of cellular bioenergetics. **Future Directions:** In this review, we describe the cross-talk between heme oxygenase-1 (HO-1) and its products with other metabolic pathways. HO-1, which we have labeled Nike, the goddess who personified victory, dictates triumph over pathophysiologic conditions, including diabetes, ischemia, and cancer. *Antioxid. Redox Signal.* 20, 1709–1722.

Heme Synthesis and Degradation Pathways in Cell Metabolism

THE HEME DEGRADATION PATHWAY is a critical and the only catalytic process that enables removal of toxic heme in cells and tissues. Heme in complex with proteins such as hemoglobin, myoglobin, cytochrome c, cytochrome p450, nitric oxide synthases (NOS), or guanylate cyclase is not dangerous for cells. The heme moiety is critical for protein function and in most, if not all, cases, oxygen and NADPH are required for their activity (1, 91). Free heme can cause oxidative injury when the iron is rapidly lost from the heme porphyrin ring and contributes in the ferrous state toward the generation of reactive oxygen species (ROS). Heme is essential for all aerobic organisms and is synthesized from protoporphyrin IX and ferrous ion (Fig. 1). Heme regulates the expression of many genes, in addition to HO-1. Heme can bind to Bach1,

a repressor protein that acts through an Maf recognition element site in the promoter of target gene which inhibits expression (120).

The first step of heme biosynthesis in eukaryotic cells is catalyzed by 5-aminolevulinate synthase (ALAS). The ALAS1 isoform is expressed in all tissues and is sensitive to the presence of heme as a negative feedback loop, while ALAS2 is restricted to erythrocytes. These enzymes catalyze the condensation step of glycine and succinyl CoA to 5-aminolevulinic acid (ALA). Heme biosynthesis is initiated in mitochondria from where ALA is transported into the cytosol where the first porphyrin ring is formed, coproporphyrinogen III. ABCB6 then transports this metabolite back into mitochondria for the final steps of synthesis, including decarboxylation of two propionate residues to protoporphyrinogen IX (Fig. 1). The final conversion of protoporphyrinogen IX to protoporphyrin IX requires oxygen and is followed by the

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FIG. 1. Heme synthesis pathway. Heme biosynthesis is catalyzed by 5-aminolevulinic acid synthase (ALAS). The first step of condensation of glycine and succinyl CoA to 5-aminolevulinic acid (ALA) occurs in mitochondria. Coproporphyrinogen III (COPRO III) is formed as a first porphyrin and transported back to mitochondria from the cytosol, where it gets converted to protoporphyrinogen IX. The final conversion of protoporphyrinogen IX to protoporphyrin IX (PPIX) and insertion of the iron atom into the ring system generates heme *b*.

insertion of the iron atom into the porphyrin ring generating heme *b*. The enzyme catalyzing this reaction is known as ferrochelatase.

There are two HOs that convert heme to biliverdin (BV) (69). In addition to heme, the HO-1 isoform is induced by oxidative stress, cytokines, heavy metals, and bacterial endotoxins. The second isoform, HO-2, is constitutively expressed in multiple organs with highest expression in the brain and testes (27, 86). Liberation of carbon monoxide (CO) uses one molecule of NADPH, while conversion of BV to bilirubin (BR) consumes an additional two NADPH molecules. NADP⁺ fuels, in turn, the pentose phosphate pathway and other anabolic processes that enable the regeneration of proton force and generate important molecules for the biosynthesis of nucleic acids and proteins (Fig. 2). HO-1 is a critical member of cellular metabolism, and its activity may influence other NADPH- and oxygen-consuming pathways, including fatty acid synthesis, oxidative metabolism of cytochrome p450, or modulation of ROS generation in phagocytes. All these pathways use a common pool of NADPH, and, therefore, acceleration of one may influence the activity of the others. Another enzyme, HO-2 contains two heme binding sites that enable its action as oxygen sensor (114).

One of the main regulators of HO-1 expression is the oxidant responsive transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Nrf2 is critical in rapidly proliferating cells and in the presence of a highly active PI3K-Akt pathway, it redirects glucose and glutamine toward anabolic pathways (75). Among the HO-1 products, CO has been best characterized and shown to promote the shuttling of glucose toward the pentose phosphate pathway rather than the tricarboxylic acid (TCA) cycle (102). However, it is likely that the other heme degradation products, including a 4th product, NADP⁺, also influence cellular metabolism.

Crosstalk between heme and anabolic pathways

Anabolic pathways that are responsible for generating proteins and nucleic acids require energy from ATP. ATP is generated primarily in mitochondria *via* oxidative phosphorylation and the electron transport chain and in the cytosol during glycolysis. Both processes can be modulated by CO (23). CO targets mitochondria and modulates the heme-containing cytochrome oxidases, resulting in an increase in ROS production and thereby enabling cytoprotective conditioning of the cell (23, 85). CO is also a competitive inhibitor of the terminal cytochrome oxidase, binding selectively to the

FIG. 2. Cross-talk of heme degradation pathway and cell metabolism. The heme degradation pathway is the only catalytic process that enables removal of toxic heme in cells and tissues. Heme is a part of hemoproteins and regulates its own synthesis. Generation of carbon monoxide (CO), biliverdin/bilirubin (BV/BR), and iron influences several cellular processes, including glucose lipid and nucleotide metabolism.

reduced binuclear heme a₃/CuB center (51). Inhibition of cytochrome oxidase results in suppression of oxidative phosphorylation and lower ATP production that corresponds to enhanced mitochondrial redox signaling. CO suppresses oxidative respiration by 12% under normoxic conditions and at a much higher rate of 70% at 1% oxygen (23, 51). In macrophages, CO-induced mitochondrial-dependent ROS stabilize p38 MAPK and peroxisome proliferator-activated receptor γ (PPAR γ) to elicit anti-inflammatory and cytoprotective effects (10, 11, 124). Such transient signaling is beneficial for cellular preconditioning and mitochondria biogenesis where cellular energy demands are increased.

Importantly, CO has been shown to increase the oxygen consumption rate (OCR) in the brain, resulting in a decrease in glucose utilization and decreased lactate production with improved oxidative phosphorylation (5). It is well established that this metabolic switch from glycolysis to anabolic synthesis and increased respiration may be an important preconditioning mechanism leading to long-term protection of the cells in response to secondary insults. Similar to neurons, we have recently found that CO increases the OCR and ROS in cancer cells that correlated with cell cycle inhibition and a concomitant increase in cell death in response to chemotherapeutics (112a). An improvement in respiration in response to CO in astrocytes was accompanied by an increase in OCR, a decrease in lactate production, and a reduction in glucose utilization (5), similar to our observation in cancer cell cultures. Overall, CO improves cellular metabolism with high ATP/ADP ratios and decreases lactate production, suggesting that even when glucose is less utilized, there is still effective ATP production (3).

Heme is a part of numerous metabolic enzymes as a core catalytic unit or as a cofactor in enzymatic processes (e.g., cytochrome p450, iNOS, and guanylate cyclase) (Table 1). Several studies show that HO-2 is important in part, in the control of both gluconeogenesis and glycolysis. HO-2-deficient mice are hyperglycemic and insulin resistant (97). A recent report suggested that HO-1 and HO-2 can interact with the glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphates 4 (PFKFB4) (62). Overexpression of HO-2 increased expression of PFKFB4, which was inhibited in responses to glucose deprivation (≤ 2.5 mM) and occurred concurrently with induction of HO-1 (62).

Cyclooxygenase (COX) is a heme protein that regulates vascular function and inflammation through the generation of prostaglandin H₂ from arachidonic acid. The cross-talk between COX and heme degradation is well established (38). Induction of HO-1 by cobalt protoporphyrin (CoPP) or adenoviral transfer of HO-1 blocks inflammation, including COX-2 expression in response to endotoxin in endothelial cells (94). Overexpression of HO-1 in endothelial cells resulted in a marked decrease in PGE₂ and 6-keto PGF₁ α levels in response to tumor necrosis factor α (TNF α) that correlated with an increase in G1 arrest (57). In the same experiments, the authors confirmed that COX activity increased in cells as measured by the levels of PGI₂ and PGE₂ in the presence of HO-1 antisense (57). CO induces COX-2 expression and PGE₂ production in macrophages in response to endotoxin (66).

Cross-talk between HO-1 and catabolic pathways

Heme degradation belongs to the category of catabolic pathways that generates small molecules and modulates signal transduction. The main catabolic pathway in cells is glucose metabolism. Carbohydrates, including glucose, are the major short-term fuel found in cells, as these are simpler to metabolize than proteins and fat when there is a need for an immediate energy source. Therefore, the control of glucose metabolism is critical for cell and tissue homeostasis (Fig. 3). Induction of HO-1 or application of CO or BR leads to inhibition of high blood glucose as well as to all diabetic-associated complications, including nephropathy, endothelial cell dysfunction, and insulin resistance (2). CO potentiates glucose-stimulated insulin secretion, which is accompanied by an increase in the acid α -glucoside hydrolases and partially dependent on iNOS and cGMP signaling (77). Similarly, application of BV or BR inhibits NADPH-dependent superoxide production and decreases glucose levels in a db/db diabetic mouse model, (30) which could be explained, in part, as an ROS scavenging mechanism or *via* direct effects on cell metabolism.

Glucose is metabolized through glycolysis to acetyl-CoA, which is an active metabolite and can be utilized in the tricarboxylic acid cycle (TCA, Krebs cycle, and citric acid cycle) to generate NADH. NADH is then utilized during oxidative phosphorylation in the mitochondria to further fuel energy consumption by the cell. There are several important

TABLE 1. HEME TYPES IN HEMOPROTEINS AND THEIR FUNCTION

Type of heme	Function of hemoprotein	Hemoprotein
Heme a	Multiheme enzyme	Cytochrome c oxidase (subunit)
Heme b	Electron transfer	Cytochrome b562
	Gas transportation/storage	Hemoglobin
	Gas transportation/storage and binding of small molecules	Myoglobin
	Binding of small molecules	CO-sensing proteins
	Enzyme	Cytochrome c peroxidase
	Enzyme	Catalase I
	Enzyme	Cytochrome P450
Heme d1	Enzyme	Mitochondrial cytochrome c
Heme P460	Multiheme enzyme (c + P460)	Cytochrome cd1 nitrite reductase
SiroHeme	Enzyme	Hydroxylamine oxidoreductase
Unusual heme	Enzyme	Sulfite reductase
		Myeloperoxidase

FIG. 3. Heme oxygenase-1 (HO-1) and metabolic effects in cells. Overall activity of metabolic pathways, both anabolic and catabolic, in the presence of active HO-1. HO-1 activity may influence other NADPH- and oxygen-consuming pathways, including fatty acid synthesis, oxidative metabolism of cytochrome p450, or modulation of reactive oxygen species (ROS) generation in phagocytes. All these pathways use a common pool of NADPH, and, therefore, acceleration or deceleration of one may influence the activity of the other.

regulatory mechanisms through which HO-1 is implicated in glucose catabolism.

In addition to regulating oxidative phosphorylation, exogenous CO exposure or HO-1-derived CO can attenuate the breakdown of glucose and significantly drive the glucose conversion metabolites into the pentose phosphate pathway, supporting a role for CO in modulating glucose biotransformation (102). The pentose pathway is an alternative pathway to the TCA cycle that enables the generation of necessary metabolites for DNA biosynthesis and of aromatic amino acids for protein biosynthesis. It is plausible that part of the modulatory effects of CO on proliferation and cytoprotection can be attributed to a shift toward generation of NADPH as a result of CO-induced ROS generation. CO targeting of mitochondrial complexes influences the generation of critical mediators during anabolic processes such as generation of ribose-5-phosphate and erythrose-4-phosphate. Similarly, the second step of heme degradation that is catalyzed by biliverdin BV reductase generates additional NAD⁺, which can also influence the pentose pathway and the TCA cycle. The data supporting this hypothesis are missing in the literature; therefore, the interaction between heme degradation and other metabolic pathways needs to be further investigated. Inhibition of the TCA cycle by blocking fumarase expression with either a selective inhibitor or in mice lacking fumarase

leads to strong induction of HO-1 (29). Lack of fumarase, which is responsible of conversion of fumarate to malate, is typical in hereditary leiomyomatosis and renal cell cancer and leads to inhibition of the TCA cycle. However, even with deletion of fumarase, cells can survive, in part, due to the compensatory induction of hypoxia-inducible factors (HIF). Stabilization of HIF in cells lacking fumarase occurs even under normoxia primarily due to induction of HO-1 and heme degradation. Frezza *et al.* found that inhibition of HO-1 in the absence of fumarase renders cells synthetically lethal (29). Treatment with the HO-1 inhibitor, ZnPP, or knockdown of HO-1 by shRNA in kidney cells enabled selective apoptosis of fumarase-negative cell clones. These studies provide the first link as to how heme degradation is fueled by heme synthesis resulting from inhibition of the TCA cycle.

Targeting Mitochondria and Cellular Respiration by HO-1-Derived CO

Energy balance and CO

CO is a therapeutic molecule that is currently being evaluated in several clinical trials (www.clinicaltrials.gov). When produced endogenously or administered exogenously in cells, it can modulate inflammatory processes, vascular homeostasis, and cellular responses to various stimuli (87). A balance between mitochondrial metabolism and cytoprotective danger-like signaling enables improvement in the majority of disease conditions after short treatment with low, nontoxic doses of CO (Fig. 4). One of the main functions of endogenous CO is to act as a diffusible messenger in cells to regulate signal transduction corresponding to the cellular need at the time. CO is a nonreactive and nonmetabolized molecule; however, by binding to hemoproteins, it can modulate cellular function, including metabolism. CO has a larger selectivity as compared with nitric oxide (NO), which unlike CO can interact with Fe²⁺ within the protoporphyrin ring as well as with nitrosylate thiol groups that can alter protein functionality (41).

One of the most well-described targets for CO is cytochrome c oxidase as described above. There are, however, multiple hemoproteins, including sGC and iNOS that can also act as targets for CO (107, 123). Recently, CO was found to increase cytochrome c oxidase activity and cellular OCR (5, 85) in astrocytes and stimulated mitochondrial biogenesis in neurons and cardiomyocytes (101) *via* the nuclear receptor PPAR γ and its coactivator 1 α (PGC 1 α) as well as Nrf2. CO effects on mitochondria biogenesis, ATP production, and regulation of COX activity are dependent on early induction of Bcl-2 protein in astrocytes. Bcl-2 is also one of the potential

FIG. 4. Metabolic pathways modulated by CO in response to tissue injury. Protective conditioning of cells after treatment with CO. CO targets mitochondrial respiration and carbohydrate metabolism to prevent damage in response to danger signals such as mechanical or pathogen-induced injury.

targets for CO (5). CO enhanced the interaction between cytochrome c oxidase and Bcl-2, which can facilitate anti-apoptotic and cytoprotective effects that are seen with CO. This effect is synergistic with CO-mediated inhibition of proapoptotic Bcl-2-related proteins in response to different types of stress (78, 122).

Increased OCR in response to CO treatment can be explained by mitochondrial uncoupling. This process occurs when energy-dissipating protons cross the mitochondrial inner membrane, resulting in a compensatory increase in O_2 consumption that is not accompanied by production of ATP. CO induces mild mitochondrial uncoupling that is an adaptation mechanism of the cells to restore altered metabolism in diseases such as cancer and diabetes (59, 67). The uncoupling effects of CO induce mitochondria biogenesis and autophagy *in vitro* and *in vivo* (59). Such changes in mitochondria explain in part the protective effects of CO in cells as well as cardiac tissue in animal models of lethal sepsis and metabolic syndrome (58, 59, 67, 89). Low levels of exogenous CO have been shown to mimic those observed with endogenous HO-1-generated CO (23). It is, however, important to consider that application of CO *in vivo* leads to immediate binding of CO to hemoglobin with a half life in humans of 2–3 h and 15 min in mice unless hyperbaric oxygen exposures are employed, which will hasten CO offloading from hemoglobin. The rapid binding of CO to hemoglobin leads to an increase in the activity of the oxygen sensors in the body, and, therefore, some of the *in vivo* effects may be explained, in part, as a pseudo-hypoxia response.

Hypoxic responses and HO-1

Ischemic injury and tissue hypoxia have perhaps the most dramatic impact on cellular physiology and metabolism. The absence of oxygen requires the cell to rapidly shift to anaerobic metabolism to ensure survival, thus rendering oxygen-seeking hemoproteins less likely to be targets for O_2 , and this would include the HOs that require O_2 for their activity. One of the major causes of cell death and organ dysfunction is ischemia or hypoxia. The reduction in inflammation after HO-1 up-regulation in response to ischemia was considered the principal protective mechanism involved in ischemia reperfusion injury (IRI) (28). For example, HO-1-overexpressing macrophages exhibit an anti-inflammatory profile and con-

tribute to the attenuation of IRI in different organs (28). More recently, the protective effects of HO-1 during IRI have been further elucidated, and additional pathways have been defined. Transcriptome analysis of animals subjected to renal IRI where HO-1 was induced led to an up-regulation of genes belonging to the vascular endothelial growth factor receptor signaling pathway family, which may, in turn, prevent ischemia by promoting local angiogenesis (22). Accordingly, Brunt and colleagues showed that *ex vivo* manipulation of Akt/HO-1 genes in human endothelial progenitor cells enhanced recovery from myocardial infarction by permitting better adaptation to tissue hypoxia (15). Corroborating these findings, Vallabhaneni showed that HO-1 adenoviral gene transfer prevented hemorrhagic shock-induced liver injury *in vivo* and decreased cellular respiration under hypoxic conditions, resulting in increased intracellular oxygen levels in the setting of low oxygen tensions (106). Moreover, HO-1 up-regulation during renal IRI enhanced the expression of genes involved in amino-acid and nitrogen metabolism, indicating a mechanism of tolerance in response to ischemia by means of a metabolic adaptation through increased amino-acid metabolism (22).

In response to tissue hypoxia, there is a dramatic increase in HIF-1 α stabilization as O_2 tensions in the cell decrease. HIF signaling seems to be a key step for HO-1 and CO-mediated cytoprotection, as it relates to modulation of the inflammatory response (10, 21). Unpublished data from our lab showed that during an ischemic episode, treatment with CO increased HIF-1 α in the kidney and correlated with improved renal function and survival after IRI (Fig. 5). Similar protection against IRI was observed in kidney allografts in pigs (40) and also in the presence of HO-1 overexpression in the infarcted porcine heart (115). These results confirm a work by Chin *et al.*, which described that CO gas increases HIF- α translational activation in macrophages (43). Perhaps the most intriguing findings in the field of IRI and tissue hypoxia were those of Mishra *et al.* (74) and Zuckerbraun *et al.* that independently showed that HO-1 and CO exposure paradoxically reduced tissue hypoxia in the lung and liver by increasing O_2 availability. In the lung, the reduction of hypoxia-induced Egr-1 signaling by CO was mediated by the blockade of ERK in a cGMP-dependent manner (74); while in the liver, HO-1 overexpression protected against hemorrhage-induced hypoxia by decreasing OCR and

FIG. 5. Effects of CO on cellular bioenergetics under normoxic and hypoxic conditions. CO differentially influences the oxygen consumption rate (OCR) under normoxic conditions, inducing protective preconditioning. In the presence of low oxygen tension, CO inhibits OCR further to sustain energy requirements, and fuel hypoxic metabolism.

increasing oxygen tensions, thus preventing bioenergetic failure (106).

CO has also been shown to potentiate lung fibrinolysis through a mechanism dependent on sGC activation; while in the liver, CO acts *via* an ROS-NF κ B pathway to prevent hepatocyte apoptosis (31, 125). Together, these data suggest that HO-1/CO have pleiotropic effects during an ischemic episode, reducing cellular inflammation and apoptosis and enhancing oxygen availability. Metabolically, this is quite astonishing given that CO has long been viewed as a molecule that would reduce O₂ availability. Quite the opposite was found when the concentrations of CO, equal to those generated by HO-1, permitted better tissue oxygenation, likely reducing the need for anaerobic metabolism and enabling the energy-requiring survival mechanisms to maintain cell and tissue stability (Fig. 5). These data meld well with the findings in the heart and muscle showing that CO increases mitochondrial biogenesis (84, 101).

BR as a Metabolite in Cells

BR as an antioxidant and its role in cell metabolism

BR, the terminal product of heme catabolism, demonstrates clear antioxidant capabilities *in vitro* (98). BR inhibits the oxidation of phosphatidyl choline in multilamellar liposomes more effectively than equimolar concentrations of vitamin E (100). BR also inhibits protein oxidation *in vitro* in the presence of a variety of oxidants, including peroxynitrite (73), superoxide, and hydroxyl radicals (79). Despite these well-characterized effects *in vitro*, the degree to which BR protects from oxidative stress *in vivo* is comparatively under-studied. Animal (25) and human (12) models of hyperbilirubinemia currently indicate a physiological antioxidant effect for BR. For example, in humans with Gilbert's syndrome (GS), a benign condition of hyperbilirubinemia, serum lipids and low-density lipoprotein (LDL) (16, 117) are resistant to *ex vivo* copper oxidation. Interestingly, when serum from GS individuals is assessed for oxidized LDL in the absence of *ex vivo* oxidation, GS individuals also possess reduced absolute oxLDL concentrations (70, 104). These data support the capacity of elevated BR to inhibit LDL oxidation. However, a recent study demonstrated that oxLDL and LDL concentrations are reduced in hyperbilirubinemic individuals. When oxLDL was expressed relative to LDL, LDL appeared more oxidized in GS compared with controls (12). Recent reports suggest that BR might not prevent physiological LDL oxidation *in vivo*, although absolute oxLDL levels are clearly reduced in hyperbilirubinemic individuals (12). Accumulation of oxidized LDL within the vascular wall and resident macrophages represents one hypothesis (of many) that might explain the development of atherosclerosis (99). Therefore, altered BR metabolism might lead to protection from atherosclerosis by preventing LDL oxidation; however, more research is necessary to verify these findings. Irrespective of whether or not BR protects from LDL oxidation, it is obvious that elevated BR (or BV) is associated with protection from multiple pathologic sequelae which are associated with oxidative stress. For example, BR or BV treatment protects against neointima formation in animals undergoing experimental angioplasty (82). In human studies, mildly elevated BR is consistently associated with preserved vascular structure (14, 42, 108), improved vascular function (70),

and a reduced risk of cardio/cerebrovascular disease (92), diabetes (39), and cancer (43, 72, 105). In addition, hyperbilirubinemic individuals have reduced concentrations of advanced glycation end products (52), indicating that BR might protect against oxidative glucose, protein, and DNA damage (76, 109).

BR clearly protects from physiological protein and thiol oxidation. In GS subjects, reduced thiol concentrations and the GSH:GSSG are greater (12, 37). Therefore, elevated BR may, in part, prevent sulfhydryl oxidation or potentially increase glutathione synthesis (33). These data are of potential interest, because BR accumulates differentially within organs (119) and could, therefore, influence cellular redox status and cell signaling *in vivo*.

BR modulates metabolic responses during inflammation

It has become evident in recent years that BR modulates the immune response. BR potently inhibits complement activation at the C1 step of the complement cascade (Bulmer *et al*; unpublished data) as well as proinflammatory cytokine production (TNF α and IL-6) and nitric oxide synthesis in experimental endotoxemia models (60, 90). Specifically, BR increases complement degradation *via* increased decay accelerating factor expression in endothelial cells (56). Elevated BR is associated with increased circulating CO and iron concentrations *in vivo* (110). These data indicate that elevated BR might stimulate a positive feedback loop of HO activation that is induced by a mild hemolytic effect (110). Elevated BR is also associated with increased circulating IL-1 β and reduced interleukin-6 concentrations, indicating novel immunomodulatory effects of BR (110). These data are supported by increased IL-1 β expression in neutrophils that are incubated with BR (113). Interleukin-6 is an activator of hepatic C-reactive protein (CRP), linking elevated BR to reduced CRP, as demonstrated in many studies (46, 118). BR is also negatively related to adiposity and is associated with reduced circulating cholesterol and triacylglycerol concentrations in humans (12, 18, 111). More recently, elevated BR in mutant hyperbilirubinemic Gunn rats was shown to induce a >50% reduction in cholesterol concentrations and reduced body mass in young female animals, providing the first evidence of a role for BR in modulating whole body metabolism (12, 111). One potential mechanism of the effects of BR on lipid lowering might depend on aryl-hydrocarbon receptor activation (83). Absence of the aryl hydrocarbon receptor (AhR) resulted in increased expression of genes regulating cholesterol biosynthesis (103). Furthermore, administration of an AhR ligand (2,3,7,8-tetrachlorodibenzo-p-dioxin) decreases the expression of cholesterol biosynthetic genes (103). Whether BR *per se* inhibits cholesterol synthesis in an AhR-dependent manner has not yet been determined. BR also inhibits triacylglycerol lipase activity and stimulates glucose oxidation in adipocytes, independent of adenylate cyclase activity and cAMP concentrations (93). These findings curiously appear related to a growing body of literature showing that elevated BR is related to reduced HBA1c (81), improved insulin sensitivity, and reduced prevalence of metabolic syndrome in healthy and diseased individuals (36, 118). BR concentrations are lower in overweight individuals and negatively associated with fasting glucose, insulin, and abdominal obesity (49). Furthermore, BR concentrations increase

FIG. 6. Interaction between bilirubin and lipid/glucose metabolism. Unconjugated BR originating from heme catabolism primarily in the spleen is glucuronidated by hepatic uridine glucuronosyltransferase 1A1 (UGT1A1) forming bilirubin glucuronides, which are excreted in the bile and involved in the digestion of fat in the intestine. Gilbert's syndrome (GS) is characterized by reduced UGT1A1 inducibility/expression and increased in circulating unconjugated BR. In adipocytes, increased unconjugated BR inhibits triacylglycerol lipase activity and increases glucose oxidation. The increase in unconjugated BR also reduces circulating cholesterol concentrations (possibly mediated by impaired cholesterol biosynthesis), and contributes to the reduced prevalence of obesity and metabolic syndrome in hyperbilirubinemic individuals.

with acute weight loss (6), suggesting that metabolism is intricately related to HO and BR metabolism (Fig. 6).

Iron and Metabolism

Free iron is the third product of heme degradation and one that induces transcription of the heavy chain of ferritin to accelerate immediate sequestration of this potential oxidant. Iron accumulation leads to liver disease and diabetes; however, iron is critical in numerous biological processes as a key component of the oxidation-reduction reactions in the mitochondrial respiratory chain as well as oxygen binding to hemoglobin. Free iron is toxic because of its participation in Fenton chemistry; reduced iron reacts with hydrogen peroxide or lipid peroxides and produces highly reactive radicals. Interestingly, a diet high in iron induces AMP kinase (AMPK) signaling and improves glucose tolerance in mice (45). Increased glucose uptake in mice fed a high iron diet was dependent on activated liver kinase B1 (LKB1) that phosphorylated AMPK. A mechanism for LKB1 activation involved changes in redox status, which resulted in a decrease in acetylation of LKB1. Importantly, glucose levels in the serum correlated with low lactate levels and decreased expression of gluconeogenic genes in the liver in animals fed a high iron diet. Similar to high iron levels in the serum in mice, serum ferritin levels in humans correlated with a decrease in the incidence of type 2 diabetic and obese subjects as compared with healthy subjects (32, 65). Increased iron or ferritin stores are positively correlated with the development of glucose intolerance and type 2 diabetes in humans. Type 2 diabetes is common in individuals with the genetic disorder hemochromatosis in which mutation of the hemochromatosis gene (HFE), which is involved in iron absorption, results in accumulation of iron in the tissues.

Proper deposition of iron in complex with ferritin is critical for homeostasis and iron metabolism. Adiponectin is one of the cytokines that regulates iron storage through induction of HO-1 in an AMPK-PPAR- α -dependent mechanism (65) and is, thus, critical for iron metabolism. Mice fed a diet high in iron or cultured adipocytes treated with iron exhibited decreased adiponectin mRNA and protein (32), which might define a negative feedback loop that regulates iron overload (65).

Heme Metabolism in the Metabolic Syndrome, Diabetes, and Cancer

Diabetes is a prototypical metabolic syndrome, while cancer can also be associated with abnormal metabolism. Diabetes and obesity are associated with elevated oxidative and inflammatory activities (enhanced level of cytokines, such as TNF, IL-6, IL-1 β , and resistin, which lead to c-Jun-N-terminal kinase [JNK] and NF κ B pathway activation). The HO system inhibits inflammation through suppression of macrophage infiltration and decreases in pro-oxidant and proinflammatory transcription factors. A common feature of metabolic syndromes is abnormal glycolysis and/or alterations in mitochondrial respiration. Otto Warburg defined what has come to be considered a common feature of cancer cells: a metabolic switch from mitochondrial respiration to anaerobic glycolysis that leads to generation of lactate from pyruvate instead of pyruvate entering the TCA cycle. Hypoxic tumors strive to survive in an environment with insufficient oxygen and nutrition while producing the majority of ATP utilizing the less efficient glycolytic pathway. Reprogramming to carbohydrate metabolism and anabolic pathways in cancer serves as an adaptation to the high proliferative rate of cancer cells and is controlled, in part, by HIF and downstream oncogenes (34). Since HO-1-derived CO is a

FIG. 7. Similarities in metabolic dysfunction in cancer and diabetes. Changes in metabolism that occur in cancer and diabetes that contribute to disease progression. The major dysfunctional metabolic pathways in cancer cells and diabetic milieu are presented.

potent regulator of HIF-1 α , it is plausible that heme metabolism can influence and participate in cancer progression (Fig. 7).

The most striking representation of the role of HO-1 and its products in metabolic disorders are through the analysis of HO-1 knockout mice and human HO-1 deficiency. Lack of HO-1 in humans is associated with growth retardation, increased iron deposition, and anemia among abnormalities in coagulation and early death (53, 116). All the disorders associated with HO-1 deficiency are due to oxidative stress and are strongly associated with metabolic syndrome. It is likely that if HO-1-deficient humans survived longer, they would develop metabolic complications such as diabetes and cancer. Similar to human HO-1 deficiency, HO-1 knockout mice that survive (survival rate ~1%–5% of litters) show high levels of oxidative stress, enlarged organs and are severely sensitive to stress and injury with a shortened life span. These null mice do not develop cancer or diabetes without additional stimuli. It is likely, however, that those mice which survive compensate with HO-2.

Metabolic syndrome is a complex clinical problem, including abnormal body fat distribution, insulin resistance, atherogenic dyslipidaemia, elevated blood pressure, as well as a proinflammatory and a prothrombotic state in the tissues (4). Each of these changes in the function of cells and tissues can be regulated by the heme degradation pathway (26) (Fig. 7). CoPP, a pharmacologic inducer of HO-1, resulted in increased adiponectin levels and improved insulin sensitivity *via* increased AMPK phosphorylation in the diabetic obese rat

model (80). Further, induction of HO-1 selectively enhanced polarization toward an anti-inflammatory M2 macrophage phenotype and reduced pericardial adiposity and cardiac injury in diabetic cardiomyopathy in obese rats (48). Similar results were reported in spontaneously hypertensive rats after treatment with hemin (61). Hemin therapy lowered blood pressure, decreased glycemia, reduced insulin resistance as well as proteinuria/albuminuria, and enhanced glucose transport. However, the direct role of HO-1 in regulating metabolism of a single cell in this scenario has not been tested.

CoPP induced HO-1 up-regulation in nonobese diabetic mice and led to a decrease in blood glucose, increased β cell survival, and decreased infiltration of CD11c+ dendritic cells (44, 63). CoPP also exerted significant HO-1-independent effects, including induction of STAT3 (71). Importantly, there is a mild association between microsatellite polymorphisms in the HO-1 gene promoter region in type 2 diabetes syndrome manifestation (7, 20). Bao *et al.* found that elevated HO-1 plasma levels were increased in newly diagnosed type 2 diabetic patients which was associated with impaired glucose tolerance (8). This study, however, did not clarify whether HO-1 is a functional protein that mediates the protection or serves as a marker of oxidative stress. Validation in HO-1 knockout mice is warranted (71).

In contrast to the diabetic milieu, the role of HO-1 in tumor metabolism and growth is much less understood (9a, 32a, 50, 80a, 95, 112, 117a). HO-1 levels are elevated in the majority of cancers; however, the activity of HO-1 varies, and, therefore, heme metabolism can be dramatically altered in the tumors. Consumption of heme contained in red meat is one of the risk factors for colorectal cancer. Heme induces cytotoxicity and ROS generation in the colon that is compensated for by hyperproliferation and hyperplasia of crypt cells (47).

HO-1 is expressed in both infiltrating leukocytes as well as in cancer cells depending on the type of tumor (Table 2). In PCa patients, nuclear and enzymatically inactive HO-1 correlated with cancer progression (88, 112a), while better survival rates were observed in colorectal cancer patients where colonic HO-1 expression correlated with lower rates of lymphatic tumor invasion (9). Overexpression of HO-1 in androgen-sensitive and androgen-insensitive PCa cell lines led to a marked reduction in cell proliferation and migration that was associated with lower metalloproteinase-9 expression (35). In a study by Li *et al.*, a combination of HO-1 overexpression in the presence of low expression levels of phosphatase and tensin homolog (PTEN) correlated with disease progression in prostate cancer patients (64).

There is further evidence that HO-1 expression plays a role in cancer incidence in humans with the discovery of a GT

TABLE 2. HEME OXYGENASE-1 EXPRESSION AND ITS ROLE IN TUMORS

Type of cancer	HO-1 localization	Role
Lung cancer	Cytoplasmic	Associated with higher stage (24)
Prostate cancer	Nuclear	Associated with disease progression (64)
Pancreatic cancer	Cytoplasmic	Proangiogenic and protumorigenic (9a)
Head and neck cancer	Nuclear	Associated with malignant progression (32a)
Glioma	High in macrophages	Proangiogenic (80a)
Colon cancer	Cytoplasmic and in macrophages	Associated with better prognosis (9)
Gastric cancer	Cytoplasmic and in macrophages	Associated with better prognosis (117a)

length polymorphism of the promoter for HO-1 which has been shown to be highly correlative with disease severity, including cancer (19, 54). Individuals with the long (GT)_n repeat and associated low expression of HO-1 showed an association with a higher frequency of gastric or lung adenocarcinoma and oral squamous cancer than those with short GT repeats and higher HO-1 expression (68). HO-1 expression in nonsmall cell lung cancer was associated with more advanced and metastatic disease (24), while others reported no correlation or decreased expression of HO-1 in macrophages in cancer specimens as compared with controls (13). Inhibition of HO-1 using an epidermal growth factor receptor inhibitor and cisplatin decreased proliferation of the lung cancer cell line A549 (24). Further, knockdown of HO-1 in A549 induced apoptosis and activation of caspase-3 as well as sensitized cells to cisplatin treatment (55) and irradiation (121). In contrast, overexpression of HO-1 was shown to inhibit lung cancer growth *in vivo* and *in vitro* through regulation of several oncomirs and angiomiRs (96). HO-1, in part, acts in lung cancer through inhibition of mir-378 and reduces tumor growth, metastases, and angiogenesis (96). There are multiple reports suggesting that overexpression of HO-1 can block or accelerate tumor growth depending on the cancer cell type. It is likely that the metabolic status of cancer cells influences how the heme degradation enzymes modulate tumor growth.

Innovation

This review provides a comprehensive discussion about the influence of heme catabolism on cellular metabolic signaling pathways. Heme is a critical component of multiple hemoproteins that are implicated in glucose, lipid, and protein metabolism. Heme turnover is tightly regulated by the heme oxygenases (HO) with the products of degradation eliciting remarkable biologic effects, including modulating signal transduction and biochemical activity. We describe the consequences of the absence of HO-1, the isoform that is critical in the stress response, and how inappropriate heme metabolism contributes to the development of pathology, including increased susceptibility to cancer, diabetes, and ischemia-reperfusion injury.

Concluding Remarks

The ability of the cell to modulate metabolic processes and adapt to a continuum of environmental cues should be dynamic. The idea of stasis is inherently flawed, as the milieu in which the organism finds itself is always and continuously challenged by the environment. We have focused this review on the role of heme, a fundamental and necessary asset of all cells, in controlling metabolism and overall cellular function. We have summarized the properties of the HO catalytic pathway and the bioactive products it generates that are intimately intertwined with cellular metabolism. It has become apparent that cellular bioenergetics, including glucose and lipid metabolism, are strongly influenced by both CO and BR. Their role in metabolic syndromes dictates the therapeutic application of both molecules in such settings. Nike was the goddess of victory and success and clearly, the success of the cell requires the elegant synchrony of heme biology and metabolism to adapt to the environment and ensure continued survival.

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Abbreviations Used

AhR = aryl hydrocarbon receptor
 ALA = 5-aminolevulinic acid
 ALAS = 5-aminolevulonate synthase
 AMPK = AMP kinase
 BR = bilirubin
 BV = biliverdin
 CO = carbon monoxide
 CoPP = cobalt protoporphyrin

COPRO = coproporphyrinogen
 COX = cyclooxygenase
 CRP = C-reactive protein
 Egr-1 = early growth response protein
 ERK = extracellular signal regulated kinase
 GS = Gilbert's syndrome
 GSH = glutathione
 HIF = hypoxia-inducible factor
 HO = heme oxygenase
 IRI = ischemia reperfusion injury
 JNK = c-jun N-terminal kinase
 LDL = low-density lipoprotein
 LKB1 = liver kinase B1
 NOS = nitric oxide synthase
 Nrf2 = nuclear factor 2
 OCR = oxygen consumption rate
 PFKFB = 6-phosphofructo-2-kinase/fructose-2,6-biphosphates 4
 PGE = prostaglandin
 PGI = prostacyclin
 PPAR γ = peroxisome proliferator-activated receptor γ
 ROS = reactive oxygen species
 STAT = signal transducer and activator of transcription
 TCA = tricarboxylic acid
 TNF = tumor necrosis factor
 UGT1A1 = uridine glucuronosyltransferase 1A1