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Review

Heme Oxygenase-1 as a Potential Therapeutic Target for Hepatoprotection

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Heme oxygenase (HO), the rate limiting enzyme in the breakdown of heme into carbon monoxide (CO), iron and bilirubin, has recently received overwhelming research attention. To date three mammalian HO isozymes have been identified, and the only inducible form is HO-1 while HO-2 and HO-3 are constitutively expressed. Advances in unveiling signal transduction network indicate that a battery of redox-sensitive transcription factors, such as activator protein-1 (AP-1), nuclear factor-kappa B (NF-κB) and nuclear factor E2-related factor-2 (Nrf2), and their upstream kinases including mitogen-activated protein kinases play an important regulatory role in HO-1 gene induction. The products of the HO-catalyzed reaction, particularly CO and biliverdin/bilirubin have been shown to exert protective effects in several organs against oxidative and other noxious stimuli. In this context, it is interesting to note that induction of HO-1 expression contributes to protection against liver damage induced by several chemical compounds such as acetaminophen, carbon tetrachloride and heavy metals, suggesting HO-1 induction as an important cellular endeavor for hepatoprotection. The focus of this review is on the significance of targeted induction of HO-1 as a potential therapeutic strategy to protect against chemically-induced liver injury as well as hepatocarcinogenesis.

Keywords: Chemoprevention, Heme oxygenase-1, Hepatoprotective agents, Hepatotoxicants, Liver damage, Transcription factors

Introduction

Heme oxygenase (HO) is the first and the rate limiting enzyme in the catabolism of heme (Maines, 2005; Maines and Gibbs, 2005) to yield equimolar amounts of biliverdin, carbon monoxide (CO) and free iron (Fig. 1). To date three isoforms of HO designated as HO-1, HO-2 and HO-3 have been identified in mammals (Maines, 1988; Maines *et al.*, 1986; McCoubrey *et al.*, 1997). HO-1 is also known as heat shock protein 32. Its human form is composed of 288 amino acids with a molecular mass of 32,800 Da and shares about 80% amino acid sequence identity with rat HO-1 (Yoshida *e al.*, 1988). On the other hand, human HO-2 is a 36 kDa protein that consists of 316 amino acids with three cysteine residues (McCoubrey *et al.*, 1992; Ishikawa *et al.*, 1995). HO-3, a 33 kDa protein, has been recently described as a weak catalyst for heme degradation and is not inducible. HO-1 is highly inducible by hemin and other none-heme agents such as ultraviolet (UV), hydrogen peroxides, heavy metals, hypoxia, and nitric oxide (Keyse *et al*., 1989; Tyrrell *et al*., 1993; Janssen *et al*., 1994; Motterlini *et al.*, 2000).

Immunochemical studies with specific monoclonal antibodies have revealed the distribution of HO-1 and HO-2 in the rat liver with distinct topographical patterns (Goda *et al.*, 1998). Thus, HO-1 has been shown to be expressed principally in Kupffer cells while HO-2 is expressed in parenchymal cells (Bauer *et al.*, 1998). Trakshel *et al*. (1986) demonstrated that under unstimulated conditions, the activity of HO-2 was 2- to 3-fold higher than that of HO-1, while the activity of HO-1 increased more than 100 fold in the presence of cadmium or cobalt. Under conditions of oxidative stress, hypoxia or hyperthermia, the induction of HO-1 would account for the majority of heme breakdown leading to the formation of bilirubin and CO.

Since HO-1 is induced as a protective mechanism in response to various stimuli, targeted induction of this stress-response enzyme may be considered as an important therapeutic strategy for the protection against inflammatory processes and oxidative tissue damage. In this article, recent findings on the implications of HO-1 induction in cellular adaptive cytoprotective response to chemical insults and

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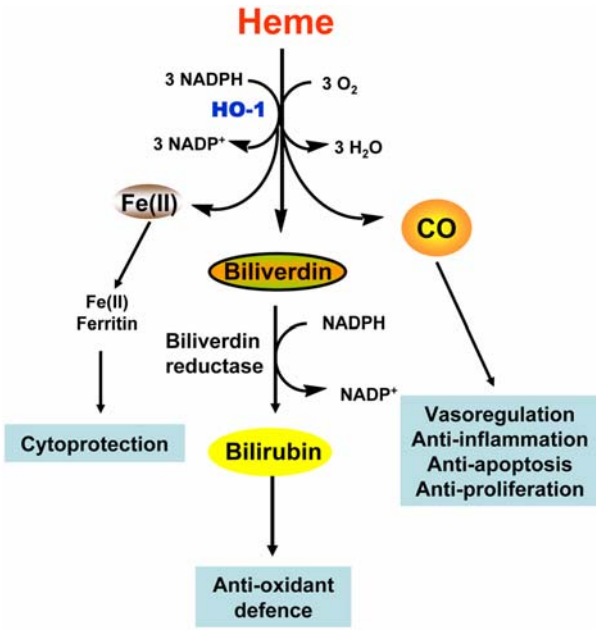

Fig. 1. Heme degradation pathway. HO degrades heme to biliverdin, carbon monoxide (CO) and iron. Biliverdin is subsequently converted to bilirubin by cytosolic biliverdin reductase. Iron is normally sequestered by ferritin, preventing it from participating in the detrimental Fenton reaction. CO produced possesses anti-inflammatory, anti-oxidant, anti-apoptotic, anti-proliferative and vasodilatory effects. Biliverdin and bilirubin are known to have potent anti-oxidant properties.

inflammatory conditions are reviewed, with particular emphasis placed on targeted HO-1 induction for hepatoprotection.

Protective Roles of HO-1 in Experimentally-induced Hepatic Damage

Induction of HO-1 has been shown to protect against a wide array of noxious stimuli, such as ultraviolet radiation, hyperoxia, lipopolysaccharide (LPS) and heme-induced damage (Alam *et al*., 1989; Motterlini *et al*., 2000, Naito *et al.,* 2004; Fuji *et al.,* 2003). In a model of ischemia-reperfusion injury, increased expression of HO-1 correlates with improved survival and organ functions in the heart, kidney and liver (Amersi *et al.,* 1999; Tamion *et al.,* 2001; Masini *et al.,* 2003). The induction of HO-1 by certain chemical compounds and some pathophysiologcal conditions is depicted in Fig. 2.

HO-1 has been shown to be protective in several disparate models of hepatic injuries. In an ex-vivo transplant model, up-regulation of HO-1 prevented ischemia/reperfusion injury (Amersi *et al.,* 1999, 2002). HO-1 induction was subsequently shown to alleviate ischemia-reperfusion injury in aged rat liver (Wang *et al.,* 2004, 2005).

Some HO-1 inducers have potential for use as effective

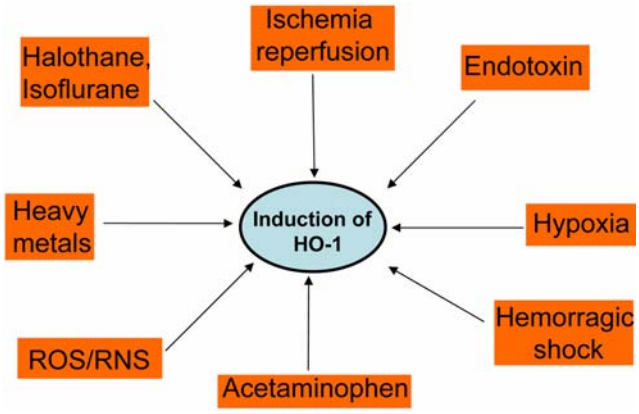

Fig. 2. The induction of HO-1 by a wide array of hepatotoxic chemicals and conditions conferring adaptive cytoprotective response.

preconditioning agents in solid organ transplantation. Curcumin, a candidate pharmacological preconditioning agent, was recently demonstrated to protect hepatocytes against oxidative injury and this protection was mediated through HO-1 induction (McNally *et al.,* 2006). In addition, curcumin pretreatment resulted in up-regulation of HO-1 during simulated cold preservation and reperfusion, which was associated with cytoprotection. Thus, curcumin may be of therapeutic benefit in liver transplantation by harnessing the stress response and allowing for prolonged duration of cold storage usually needed for hepatic allografts during the transplantation process.

Pannen *et al.* (1998) demonstrated that HO-1 was hepatoprotective in a systemic model of rat hemorrhagic shock and resuscitation. In addition, over-expression of HO-1 has been shown to be protective against endotoxin mediated hepatic dysfunction (Kyokane *et al.,* 2001; Dorman *et al.,* 2004). In addition, Wunder *et al*. (2002) demonstrated that in remote organ injury initiated by 1-h bilateral hindlimb ischemia followed by either 1- or 1.5-h reperfusion (I-R) in male C57BL/6 mice, the induction of HO-1 reduced leukocyte accumulation within liver sinusoids and postsinusoidal venules. Furthermore, this study demonstrated that suppression of HO activity by chromium mesoporphyrin, a competitive inhibitor of HO, elicited a significant increase in leukocyte adhesion within postsinusoidal venules and elevation of sinusoidal plugging by leukocytes (Wunder *et al*., 2002). Induction of HO-1 in protecting human hepatocytes against hypoxia has recently been demonstrated, suggesting that HO-1 induction may have therapeutic potential against inflammatory insults (Tuzuner *et al.,* 2004).

Further evidence underscoring the role of HO-1 in cellular defense came from investigations using mice deficient in HO-1. Adult mice deficient in HO-1 were more susceptible to hepatic necrosis, and developed anemia when subjected to endotoxin (Poss *et al.,* 1997; Takahashi *et al.,* 2003). The HO-1 knock-out mice also show growth retardation, and their kidneys and livers elicit iron deposition and hepatospenomegaly

and periportal inflammation (Wunder and Porter, 2003). A single case of human HO-1 deficiency was reported (Yachie *et al.,* 1999). This patient exhibited growth retardation and persistent anemia, and eventually died of intracranial hemorrhage at 6 years (Kawashima *et al.,* 2002).

The role of HO-1 in immune-mediated liver injury has also been demonstrated. Sass *et al*. (2003) found that up-regulation of endogenous HO-1 rescued mice from apoptotic liver damage induced by anti-CD95 antibody (Ab) or D-galactosamine in combination with anti-CD3 Ab, LPS, or tumor necrosis factor α (TNF-α). In addition, overexpression of HO-1 by adenoviral gene transfer ameliorated apoptotic liver damage, and during the systemic inflammatory response syndrome (McCarter *et al.,* 2004), thus providing a therapeutic modality for inflammatory liver diseases. Although, the mechanistic basis of the hepatoprotective role of HO-1 has not been fully elucidated, the products of this enzyme, namely bilverdin/ bilirubin, CO, and ferritin, have been considered to be involved.

Cytoprotective Effects of HO-1 Products

Biliverdin and bilirubin. Although biliverdin and bilirubin, the metabolites of heme produced by HO, were used to be regarded as useless or toxic products, they are now recognized as important endogenous antioxidants (Fig. 1). Antioxidant activities of biliverdin and bilirubin have been demonstrated both *in vitro* and *in vivo* (Stocker *et al.*, 1987a b; Llesuy and Tomaro, 1994). Recent studies have revealed the potent cytoprotective effects of biliverdin against hepatic ischemia reperfusion injury in rats (Fondevila *et al.,* 2004). Under certain experimental conditions, bilirubin was found to be as potent as α-tocopherol in scavenging peroxyl radicals in vitro (Stocker *et al.*, 1987b). Bilirubin at nanomolar concentrations has been shown to rescue cells from hydrogen peroxide-induced damage (Baranano *et al.,* 2002). Studies by Ossola and colleagues (1997) suggested inverse correlation between HO induction and oxidative stress in rat liver. In their study, administration of bilirubin significantly suppressed oxidative stress in rats treated with copper sulfate. The antioxidant activity of bilirubin and HO-1 induction was further demonstrated in rat liver exposed to UVA (Ossola and Tomaro, 1998). Bilirubin also elicited an antioxidant effects in rat hepatoma AH 136B primary cells. (Tanaka *et al.,* 2003; Fang *et al.,* 2004).

Carbon monoxide. CO, a gaseous by-product of heme metabolism, is produced at relatively high levels in injured tissues via induction of HO-1, and contributes to the attenuation or resolution of pro-inflammatory processes. CO has potent protective effects on acute and chronic inflammation (More *et al.,* 2005). CO suppresses the pro-inflammatory response while it enhances the anti-inflammatory function of macrophages (Yachie *et al.,* 1999). Thus, CO was demonstrated to attenuate the inflammatory response induced by LPS in RAW264.7 murine macrophages (Sawle *et al.,* 2005) and also in rat liver via modulation of iNOS expression and NO production (Sarady *et al.,* 2004; Sawle *et al.,* 2005). Moreover CO inhibits platelet activation and aggregation, thereby suppressing thrombosis and the pro-inflammatory damage induced by activated platelets (Brune and Ullrich, 1997). Other anti-inflammatory effects elicited by CO include down-regulation of the expression in macrophages of plasminogen activator inhibitor type 1 and prevention of apoptosis in several types of cells, such as endothelial cells, fibroblasts and hepatocytes (Brouard *et al*. 2002; Petrache *et al.,* 2000; Otterbein *et al.,* 2003).

CO has been shown to have potent vasoactive properties. Thus, CO was demonstrated to stimulate soluble guanylate cyclase, leading to increased production of cGMP. Through this process CO can alter smooth muscle cell activity within the wall of vessels thereby causing vasodilation (Pannen *et al.,* 1998). Suematsu *et al.* (1995) demonstrated that CO maintained a low vascular tone of liver sinusoids via cGMP-mediated relaxation of hepatic stellate cells, an effect that is particularly eminent when HO-1 gene is induced upon oxidative stress (Bauer *et al.,* l996). Furthermore, CO has been shown to affect vascular resistance indirectly by inhibiting the cytochrome P450-mediated production of endothelin-1, a vasoactive compound known to increase sinusoidal resistance (Coceani *et al.,* 1997). Studies have shown that, cGMP or p38 mitogen-activated protein kinase (MAPK) plays a role in mediating the cytoprotective effects of CO. For instance, cGMP, was shown to be involved in the antiapoptotic effects of CO in fibroblasts (Otterbein *et al.,* 2003). The anti-thrombotic effect of CO in platelets requires both cGMP and p38 MAPK (Petrache *et al.,* 2000). By utilizing an ex-vivo model of CO exposure, Amersi *et al*. (2002) found that the p38 MAPK signaling pathway could represent a key molecular mechanism by which CO protects against hepatic I/R insult.

Fe^{2+} and ferritin. The oxidation of heme by HO-1 leads to the production of iron (Fe^{2+}). Generally, Fe^{2+} even at a low concentration is known to cause cytotoxicity by catalyzing the production of hydroxyl radicals but studies have shown that the level of ferritin, the Fe^{2+}-sequestering protein, is elevated when HO-1 is induced, and decreased when HO activity is suppressed (Eisenstein *et al.,* 1991). Other studies have also shown that the ATPase pump that actively removes intracellular iron from the cell is increased in tandem with the expression of ferritin, and in this process the intracellular pool of Fe^{2+} is decreased (Ferris *et al.,* 1999). The relative contribution of the ATPase pump and ferritin to the overall cytoprotective effects of HO-1 has not been clarified, but both entities appear to play pivotal roles in removing Fe^{2+} from the cell when HO-1 expression is elevated.

Although increased ferritin levels are often observed in conjunction with HO-1 induction, the cytoprotective effects of HO-1 do not necessarily correlate with expression of ferritin. For instance, in rats subjected to endotoxic shock, HO-1

induction was shown to be ferritin independent (Otterbein *et al.,* 1997), while in an oxidative stress-induced cytotoxicity model, HO-1 induction and cytoprotection were found to be ferritin-dependent (Balla *et al.,* 1992). In spite of the controversy concerning the role of ferritin as a cytoprotective agent resulting from HO induction, recent studies continue to favor the notion that expression of ferritin is indeed related to HO activity. Hydrogen peroxide-mediated toxicity in cultured human endothelial cells (ECV 304) was accompanied by a significant induction of HO activity and ferritin synthesis (Grosser *et al.,* 2004). Gonzales *et al*. (2005) demonstrated that the induction of HO-1 by cobalt chloride in rat liver preceded an increase in ferritin and ferritin-bound iron. A significant correlation was observed between the increased levels of HO-1 and ferritin in patients with non-alcoholic fatty liver disorders (Malaguanera *et al.,* 2005).

Signal Transduction Network for HO-1 Gene Regulation

A number of intracellular signaling molecules have been identified to be involved in regulating the induction of HO-1 (Fig. 3). Some of the major upstream protein kinases and their downstream transcription factors responsible for the up-regulation of HO-1 are discussed below.

Roles of upstream kinases in signal transduction mediating HO-1 induction. Among upstream signaling kinases, extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK have been considered to play major roles in controlling up-regulation of HO-1. Activation of either one or more of these MAPKs by external stimuli triggers HO-1 gene expression. ERK1/2 and p38 MAPK are involved in the induction of HO-1 gene transcription by sodium arsenite in the hepatoma cell line (Elbirt *et al.,* 1998).

Other signaling enzymes that are likely to be implicated in HO-1 induction include protein kinase (PKC), protein kinase A (PKA), and phosphatidyl inositol 3-kinase (PI3K). A number of studies have recently demonstrated the role of PKC in the regulation of HO-1 gene expression (Li *et al.,* 2004; Numazawa *et al.,* 2003). Intracellular levels of cAMP are elevated by a large number of hormones and external stimuli, resulting in the activation of PKA. Immenschuh *et al*. (1998) reported that treatment of primary rat hepatocytes with the PKA-stimulating agents, such as Bt_2cAMP and glucagon, resulted in a dose-dependent induction of HO-1. In contrast, Bt_2cAMP and glucagon inhibited HO-1 induction by cobalt (Co^{2+}) in chick embryo hepatocytes, suggesting that PKA-dependent induction of HO-1 is cell- or stimuli-specific. In addition, cGMP formed via activation of soluble guanylate cyclase, either directly by NO-releasing agents or by the induction of inducible nitric oxide synthase (iNOS), has been shown to up-regulate HO-1 gene expression (Polte *et al.,* 1997; 2000).

The PI3K/Akt pathway controls the intracellular levels of ROS by regulating the expression of HO-1 (Salinas *et al.,* 2003). A recent study from our laboratory also suggested the involvement of PI3K/Akt signaling pathway in the induction of HO-1 by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) in human mammary cancer (MCF-7) cells (Kim *et al.,* 2004). The PI3K-mediated activation of nuclear factor E2-related factor-2 (Nrf2) has been proposed to be a major signaling

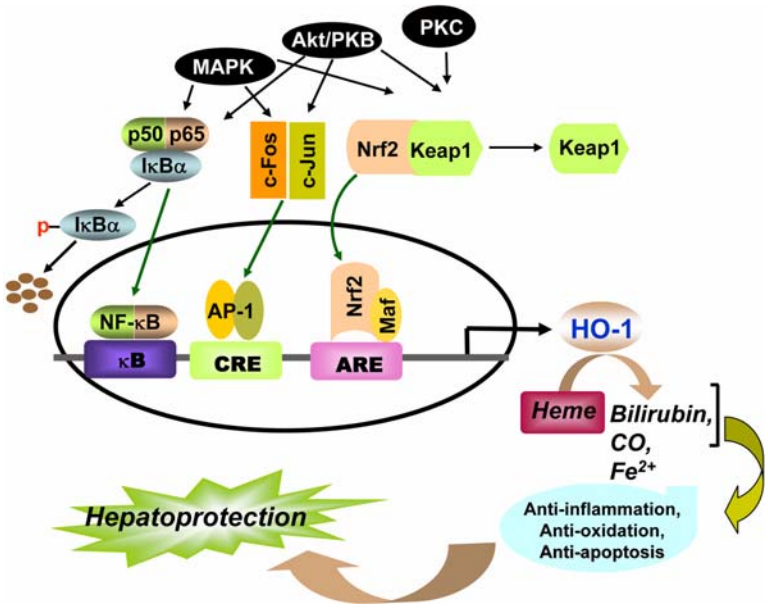

Fig. 3. Regulation of HO-1 by transcription factors and their upstream kinases. Under normal conditions, transcription factors, such as NF-κB, AP-1 and Nrf2, are located in the cytosol. Upon external stimuli, the active forms of these transcription factors translocate to the nucleus where they bind to the specific DNA sequence leading to the transcription of HO-1 gene.

pathway in HO-1 induction by hemin in human neuroblastoma SH-SY5Y cells (Nakaso *et al.,* 2003). Besides its role in regulating HO-1 expression at the transcriptional level, Akt/PKB has also been shown to induce posttranslational modifications of HO-1 via phosphorylation of its serine-188 residue (Salinas *et al*., 2004).

Roles of transcription factors in HO-1 induction. Activator protein (AP)-1 is a major transcription factor that can transactivate HO-1 by binding to the promoter region of the gene. AP-1 is a dimeric combination of basic leucine zipper (bZIP) proteins of the Jun and Fos family, Jun dimerization partners and the closely related activating transcription factor sub-families (Karin *et al.,* 1997). Several studies have demonstrated that the induction of HO-1 by oxidative stimuli, such as heme (Shan *et al.,* 2004), sodium arsenite, cobalt chloride (Lu *et al.,* 2000), and cobalt protoporphyrin (CoPP) (Shan *et al.,* 2004), is mediated via AP-1 activation.

Nuclear factor-κB (NF-κB) has also been implicated in the induction of HO-1 expression in response to diverse stimuli, such as hemin, cadmium (Chen *et al.,* 2004), and LPS (Wijayanti *et al.,* 2004) as the inhibition of NF-κB leads to attenuation of HO-1 induction by these agents (Liu *et al.,* 2004). In addition, overexpression of NF-κB in human hepatoblastoma-derived HepG2 cells resulted in an increased HO-1 mRNA level (Lavrovsky *et al*. (2000).

Nrf2, a 66-kDa protein and a member of the cap'n'collar family of bZIP transcription factors, has been shown to play an essential role in the ARE-mediated expression of phase II detoxifying, antioxidant, and stress-inducible genes including HO-1 (Itoh *et al.,* 1997). Nrf2 is sequestered in the cytoplasm as an inactive complex with its cytosolic repressor Keap1. Dissociation of Nrf2 from the inhibitory protein Keap1 is a prerequisite for nuclear translocation of this transcription factor. After forming a heterodimer with small Maf protein, the active Nrf2 binds to *cis*-elements having common core sequences, alternatively known as Maf recognition element (MARE), ARE, or electrophile/stress response elements (EpRE/StRE) (Lee and Surh, 2005), leading to the expression of a battery of target genes, including HO-1.

Protective Role of HO-1 in Chemically-induced Liver Damage

Acute liver damage continues to pose serious problems in critical care due to a high rate of mortality (Gill and Sterling, 2001). Although viral hepatitis especially hepatitis B virus has been suspected to account for the majority of the liver cancer cases, but exposure to drugs and aflatoxin B_1-contaminated foods have also been implicated in the etiology of human hepatocarcinogenesis (Gill and Sterling, 2001). Numerous chemicals including acetaminophen, carbon tetrachloride and heavy metals have been classically used to induce hepatotoxicity in laboratory animals (Aleksunes *et al.,* 2004). Expression of HO-1 and other hepatic antioxidant and detoxification genes is induced in rat liver as a consequence of chemical injury (Chiu *et al.,* 2002; Nakahira *et al.,* 2003). The representative hepatotoxicants and the role of HO-1 in protecting hepatic lesions caused by these chemicals are described below.

Acetaminophen. Acetaminophen is considered a relatively safe analgesic drug at a therapeutic dose but an overdose has been shown to cause hepatic damage in experimental animals and humans (Laskin *et al.,* 1995; Makin and Williams, 1997). More than 50% of all cases of acute liver failure in the United States from 1997 to 2002 have been shown to result from exposure to drugs, and 40% of these have been attributed to acetaminophen ingestion (Lee, 2003). Acetaminophen is bioactivated by cytochrome P450 enzymes, particularly the 2E1 isoform, to *N*-acetyl-p-benzoquinone imine (NAPQI) (Nelson, 1990). NAPQI is a highly reactive electrophile that covalently binds to cellular macromolecules causing toxicity. Hepatic and non-hepatic parenchymal cells have been shown to produce ROS which have been implicated in acetaminophen-induced tissue damage (Gardner *et al.,* 1998; Michael *et al.,* 1999). It has been reported that expression of HO-1 is rapidly up-regulated in the liver following administration of toxic doses of acetaminophen (Bauer *et al.,* 2000; Noriega *et al.,* 2000). Up-regulation of HO-1 and multidrug resistance-associated proteins by a hepatotoxic dose of acetaminophen has been recently reported (Aleksunes *et al.,* 2005). Chiu *et al.* (2002) demonstrated a time dependent induction of HO-1 in the liver following treatment of rats with a toxic dose of acetaminophen, and pretreatment with hemin prevented acetaminophen hepatotoxicity. In another study, treatment of rats with acetaminophen induced HO-1 and its mRNA transcript, and inhibition of HO activity by tin protoporphyrin (SnPP) abrogated acetaminophen-induced hepatic neutrophil accumulation and NF-κB activation (Bauer *et al.,* 2000).

Nrf2 has been shown to play an important role in the detoxification of acetaminophen. Studies by Chan *et al.* (2001) revealed that mice deficient in Nrf2 are more prone to acetaminophen-induced hepatotoxicity. A direct evidence for the Nrf2 activation following acetaminophen treatment was recently demonstrated in vivo (Goldring *et al.,* 2004). According to this study, there was a dose dependent increase in nuclear levels of Nrf2 in the liver which was consistent with a pronounced nuclear translocation of this transcription factor.

Carbon tetrachloride. It is generally thought that carbon tetrachloride (CCl_4) hepatotoxicity is due to a reactive intermediate trichloromethyl radical which is generated by its reductive metabolism by hepatic cytochrome P450. In the presence of oxygen, the trichloromethyl radical forms trichloromethyl peroxy radical species which preferentially causes lipid peroxidation resulting in the breakdown of cellular membranes (De Groot and Sies, 1989). Studies have shown that HO-1 is induced in the liver of rats treated with CCl_4. In a rat model of acute CCl_4-induced hepatic injury,

Schiaffonati and Tiberio (1997) demonstrated an increase in the steady-state levels of mRNAs for HO-1, Mn and Cu/Zn superoxide dismutases and H and L ferritin subunits. In this study, induction of c-*fos* and c-*jun* protooncogenes is the earliest event after CCl_4 administration providing evidence that activation of specific stress response genes is primarily related to the defense against the rapidly occurring cell damage. Subsequently, Nakahira *et al*. (2003) demonstrated a marked increase in hepatic HO-1 expression both at transcriptional and translational levels in hepatocytes, especially around the central vein. Parallel with the induction of HO-1, there were significant increases in the hepatic malondialdehyde content, severe liver cell injury, and elevated hepatic TNF-α mRNA expression and DNA binding activity of NF-κB. These findings suggest that induction of HO-1 following CCl_4 treatment is an adaptive response which can confer hepatoprotection against further oxidative damage.

Halothane and Isoflurane. Halothane and isoflurane are anesthetic agents used in major hepatic surgeries such as liver transplantation, in which ischemic injury to the liver is a major obstacle (Chapin and Newland 1989). Halothane has been shown to cause hepatic injury including severe hepatitis (Kenna and Jones, 1995) whereas isoflurane is considered to be less hepatotoxic since it undergoes quantitatively much less metabolism to form reactive metabolites and also preserves a better hepatic blood flow (Kenna and Jones, 1995).

The metabolism of halothane is considered to occur via two different pathways both of which are catalyzed by microsomal cytochrome P450, especially the 2E1 isoform (Kenna and Jones, 1995). Halothane is metabolized under normoxia to trifluoroacetyl chloride, an unstable and reactive intermediate that induces covalent trifluroacetylation of several proteins of the endoplasmic reticulum leading to hepatic damage (Kenna and Jones, 1995). Under hypoxic conditions, however, halothane is metabolized by a reductive pathway to produce a free radical intermediate which initiates lipid peroxidation and hepatic damage (Awad *et al.,* 1996). In the halothane-hypoxia model, induction of HO-1 mRNA and its protein product occurred principally in the hepatocytes around the central vein with concomitant increase in serum alanine transaminase activity (Odaka *et al.,* 2000). In addition, hemin pretreatment induced hepatic HO-1 which abrogated the halothane-induced hepatotoxicity in this model. In contrast, the inhibition of HO activity by pretreatment with the HO-1 inhibitor, tin mesoporphyrin (Sn-MP) abolished the effect of hemin, lending further support for the implications of HO-1 induction in the protection of hepatic damage by halothane-induced hypoxia (Odaka *et al.,* 2000).

Yamasaki *et al*. (2001) reported that under hypoxic conditions, isoflurane failed to induce HO-1, suggesting that much less heme is formed by isoflurane-hypoxia whereas halothane-hypoxia induced HO-1 significantly. Their study demonstrate differential effects of isoflurane and halothane on the expression of HO-1 under hypoxic conditions and that isoflurane may cause hepatic damage to a lesser extent than halothane justifying the notion that isoflurane may be a safer anesthetic compared with halothane. In agreement with the above finding, recent studies have demonstrated the ability of isoflurane to induce HO-1 activity and expression under normoxia (Hoetzel *et al.,* 2002; Shmidt *et al.,* 2004; Buzalel and Batlle, 2005). Schmidt *et al*. (2004) demonstrated specific over-expression of HO-1 mRNA and protein in the liver, followed by an elevated HO enzyme activity in rats treated with isoflurane. This effect reduced portal venous resistance. In addition, HO inhibition by SnPP augmented portal resistance in isoflurane pretreated animals which was associated with the increased portal pressure and the reduced portal flow. These findings may implicate the HO-1 induction in therapeutic potential of isoflurane as an anesthetic agent. In mechanistic terms, Hoetzel *et al*. (2006) recently elucidated the underlying mechanisms of HO-1 induction by isoflurane. It was found that the compound induced HO-1 specifically in the pericentral region of the liver and the induction depended on the Kupffer cell function, PKC and phospholipase A_2.

Heavy metals. HO-1 can be induced in a wide range of animal tissues, particularly liver following a number of stressful stimuli including heavy metals (Dorman *et al.,* 2005).

The effects of heavy metals such as cobalt chloride ($CoCl_2$) and cobalt protoporphyrin (CoPP) on the transcription of the HO have been examined using cDNA for rat HO as a probe (Lin *et al.,* 1990). $CoCl_2$ increased HO mRNA by 40-to 60-fold after 2 h of metal exposure and this increase was prevented by co-administration of actinomycin D or cycloheximide, indicating that up-regulation of HO requires de novo protein synthesis which was regulated at the transcriptional level. Furthermore, the induction of chicken HO-1 by $CoCl_2$ was reported to be regulated by AP-1 elements (Lu *et al.,* 2000). $CoCl_2$ is known to produce oxygen-derived free radicals which leads to oxidative damage (Christova *et al.,* 2002) resulting in over-expression of HO-1 as a compensatory reaction (Kaliman *et al.,* 2001). Gonzales *et al.,* 2005 demonstrated an increase in lipid peroxidation and a decrease in GSH synthesis with concomitant induction of HO-1 expression and activity following treatment of rats with $CoCl_2$. $CoCl_2$ elicited over-expression of HO-1 which protected the rat liver from ischemia/reperfusion injury with extended cold preservation (Kato *et al.,* 2001). Sass *et al*. (2003) demonstrated that up-regulation of endogenous HO-1 by CoPP rescued mice from immune-mediated apoptotic liver damage. CoPP treatment also protected isolated primary hepatocytes from anti-CD95-induced apoptosis.

Arsenic as arsenite has been shown to induce the activity and expression of HO-1 in experimental animals (Brown *et al*, 1997; Kitchin *et al.,* 1999), in the chicken hepatoma cell line (Gabis *et al.,* 1996), human hepatoma cells (Mitani *et al.,* 1990) and chick embryo liver cells and hepatocytes (Lu *et al.,* 1997; Jacobs *et al.,* 1999). In mice treated with sodium arsenite, maximal increases of hepatic HO-1 and plasma total

bilirubin occurred at 24 h with concomitant decreases in the total hepatic cytochrome P450 monooxygenase content and catalytic activities (Suebert *et al.,* 2002). Arsenite was reported to activate MAPKs (Liu *et al.,* 1996; Cavigelli *et al.,* 1996), potently stimulates AP-1 transcriptional activity and efficiently induces c-*fos* and c-*jun* gene expression (Cavigelli *et al.,* 1996). In a chicken hepatoma cell line (LMH), sodium arsenite induced the activity of MAPKs, such as ERK, JNK and p38, which appeared to be regulated by an AP-1 element (Elbirt *et al.,* 1998). This study implicates the role of MAPK signaling cascades in sodium arsenite-mediated induction of HO-1 gene expression.

Cadmium (Cd), a representative environmental heavy metal, has been shown to be a potent inducer of a number of stress proteins including HO-1 in various animal models as well as in transgenic mice expressing the luciferase reporter gene (Abe *et al.,* 2000; Malstrom *et al.,* 2004). Treatment of primary rat hepatocytes or hepatoma cells with $CdCl_2$ (10 mM) or $CoCl_2$ (300 mM) induced HO-1 mRNA expression in parallel with 23 kDa heme-binding protein (HBP23) (Immenschuh *et al.,* 1995). The upstream stimulatory factor (USF) binding site that is functional in transcription of the HO-1 gene was demonstrated to play an important role in the induction of rat HO-1 expression by cadmium (Maeshima *et al.,* 1996). By using the yeast two-hybrid assay, He *et al*. (2001) identified activating transcription factor (ATF) 4 as a potential Nrf2-interacting protein and confirmed association between Nrf2 and ATF4 in mammalian cells by co-immunoprecipitation and mammalian two-hybrid assays. Furthermore, the Nrf2-ATF4 dimer was found to bind to ARE that harbors the consensus sequence from the HO-1 gene. This study revealed that increased expression of ATF4 in mouse hepatoma cells and detectable induction of ATF4 protein by $CdCl_2$ preceded HO-1 induction, suggesting that ATF4 regulates basal and $CdCl_2$-induced expression of the HO-1 gene.

Furthermore, Cd-induced HO-1 expression was mediated by MAPKs and transcription factors such as AP-1 and NF-κB (Elbirt *et al.,* 1998; Hart *et al.,* 1999; Liu *et al*., 2002). Liu *et al*. (2002), by using wild-type and metallothionein-I/II-null mice models, showed the activation of JNK1 and JNK 2 as well as ERK1/2 in MT-null mice following administration of Cd. In addition, AP-1 was remarkably activated after Cd intoxication in wild-type mice.

HO-1 as a Potential Target of Hepatoprotective Agents

Although significant progress has been made in the management of liver diseases, acute liver failure still remains a serious problem (Takahashi *et al.,* 2004). Liver transplantation which is currently employed in the treatment of hepatic failure is still a limited way of therapy with many drawbacks such as exorbitant costs and shortage of donors. Presently there have been limited strategies to protect liver cells from damage and degeneration. In view of the increasing experimental evidence demonstrating the antioxidant and anti-inflammatory effects of HO-1 products, the induction of this enzyme or its catalytic activity by either natural or synthetic compounds may represent an effective strategy to intervene in liver carcinogenesis and other hepatic disorders.

A wide variety of chemopreventive agents eliciting cytoprotective, anti-inflammatory, and antioxidant effects via HO-1 induction have been recognized. While continuous efforts should be geared towards searching for novel hepatoprotective agents targeting HO-1, some synthetic or naturally occurring substances presently identified to induce HO-1 as part of their chemoprevention/chemoprotection against hepatocarcinogenesis or other liver disorders are listed below.

Oltipraz and structurally related dithiole thiones. Oltipraz [4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione] and related dithiolethiones exert their chemopreventive activities by enhancing the expression of carcinogen detoxification and antioxidant genes (Kensler, 1997; Roebuck *et al.,* 2003). Oltipraz was shown to protect against acute toxicities mediated by carbon tetrachloride, acetaminophen, allyl alcohol, and aflatoxin in mice, hamsters and rats (Kwak *et al.,* 2004). The chemopreventive effects of oltipraz and related thiols have been attributed to their ability to reduce the formation of reactive metabolites of chemicals and/or to enhance their detoxification (Kwak *et al.,* 2004).

According to Primiano *et al*. (1996), treatment of rats with 1,2-dithiole-3-thione resulted in a significant increase in hepatic HO-1 activity that corresponded to increased HO-1 protein levels. Nrf2 plays a central role in regulating the induction of HO-1 and other multiple phase 2 and antioxidative enzymes by chemoprotective dithiolethiones in vivo (Petzer *et al.,* 2003; Kwak *et al.,* 2004). Petzer *et al*. (2003) reported that a major metabolite of oltipraz, when treated to murine hepatoma cells containing a luciferase gene under the control of the ARE from the mouse HO-1 gene, induced the luciferase activity, to a greater extent than that achieved with the parent compound. Western blots of nuclear proteins isolated from Hepa1c1c7 cells indicated a stimulation of nuclear translocation of Nrf2 from the cytosol (Petzer *et al*., 2003).

Organosulfur compounds in garlic. Some organosulfur compounds such as diallyl sulfide, diallyl disulfide and diallyl trisulfide present in garlic have been shown to possess pronounced chemoprotective and chemopreventive properties (Herman-Antosiewicz *et al*., 2004). Certain garlic-derived organosulfur compounds can inhibit metabolic activation of carcinogens, thereby blocking initiation of carcinogenesis (Milner, 2001; Yang *et al.,* 2001). In addition, the induction of antioxidant and phase II detoxifying enzymes by organosulfur compounds has been reported (Guyonnet *et al.,* 1999; Sheen *et al.,* 1999). Since allylsulfides undergo metabolic conversion to form sulfone derivatives (Germaine *et al.,* 2003) that may act as electrophiles, these organosulfur compounds are

thought to target cysteine sulfhydryl of Keap1, thereby activating Nrf2-regulated gene transcription and ARE activity. In support of this hypothesis, Chen *et al*. (2004) recently reported that treatment of human hepatoma HepG2 cells with various organosulfur compounds resulted in Nrf2 activation leading to the induction of HO-1. Nrf2-mediated HO-1 induction by diallyl sulfide in HepG2 cells has also been reported by Gong *et al*. (2004). According to this work, diallyl sulfide-induced HO-1 gene expression was accompanied by a transient increase in ROS production. Treatment of HepG2 cells with N-acetyl-L-cysteine, a ROS scavenger, blocked diallyl sulfide-induced ROS production, ERK activation, nuclear translocation of Nrf2, and subsequently HO-1 expression. However, the same study revealed that diallyl trisulfide-induced ARE activity was mediated via Ca^{+2}-dependent signaling, but not that of MAPKs or PKC (Chen *et al*., 2004). Therefore, the differential effects of garlic-derived organosulfur compounds on the MAPK-mediated activation of Nrf2 and HO-1 induction may be due to structural differences in terms of the number of the sulfur moiety and the length of alkyl side chain, which may confer varying degrees of electrophilicity to parent compounds as well as their active metabolites (Prawan *et al.,* 2005).

Sulforaphane. The isothiocyanate sulphoraphane, a constituent of broccoli and some other cruciferous vegetables, has been intensively investigated for its bifunctional chemopreventive effects characterized by inhibition of phase I enzymes and induction of phase II enzymes (Langouet *et al.,* 2000; Munday and Munday, 2004). The compound also increases the binding of Nrf2 to ARE, resulting in the induction of a battery of phase II detoxification genes (Zhang *et al.,* 2003). Recent studies by Jeong and colleagues (2005) using HepG2 cells demonstrated that sulphoraphane strongly induced expression and ARE-mediated transcriptional activation of Nrf2, retarded degradation of Nrf2 through inhibition of Keap1, and induced the expression of HO-1.

Conclusion

There has been increasing evidence supporting that HO-1 is protective in several disparate models of hepatic stress. The metabolic products of HO-catalysed heme breakdown have been shown to play pivotal role in cellular defense including hepatoprotection. Furthermore, the up-regulation of HO-1 expression, mediated through signal transduction network involving AP-1, NF-κB, Nrf2 and MAPKs, points to the central role of these signaling molecules in the maintenance of cellular redox homeostasis. As induction of HO-1 expression may hence hold therapeutic promises, continuous efforts towards identifying novel hepatoprotective antioxidant/anti-inflammatory substances that target HO-1 and establishment of well designed in vivo models properly evaluating the efficacy of these agents will be warranted.

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