SEMINAL HEME OXYGENASE IN FERTILE AND INFERTILE MALES

Thesis

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by

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Contents

Introduction

Heme oxygenase (HO-1) enzyme controls the initial and rate-limiting step in heme catabolism, cleaving heme to biliverdine then to bilirubin. Iron is released when the heme ring is opened, while CO is liberated. Therefore, HO enzyme down-regulates all cellular homoproteins. Heme molecule plays a centarl role in diverse biological processes in different systems, in cell respiration, energy generation, growth differentiation processes, oxidative biotransformation and generating inflammatory mediators as eicosaoids and NO synthase (NOS) ***(Suematsu et al., 2003; Mancuso et al.2004)***. The finding of high-level expression of HO-2 in cell populations not involved in haemoglobin heme degradation, such as neurons and germ cells, led to the suggestion that it has function(s) aside from heme degradation ***(Ewing & Maines, 1995)***.

All products of HO activity are physiologically active; iron, in addition to synthesis of heme, is considered to be a gene regulator. CO has been shown to activate soluble guanylyl cyclase (sGC) implicated in a variety of physiological functions ranging from neuroendocrine modulation to control the smooth muscle tone (***Naito, 2008***). Biliverdin and its reduction product, bilirubin, have antioxidant activities ***(Maines, 2000)***. In addition,

both the catalytic activity of the HO system and its products, CO and iron, affect generation of NO ***(Abraham et al., 1985)***.

Two forms of heme oxygenase, HO-1 and HO-2, were identified in the human testis. HO-2 was the predominant form; its expression is regulated at the transcriptional and translational levels ***(McCoubrey et al., 1995)***. ***Greene et al. (1991)*** demonstrated that increases in HO enzyme activity were of similar magnitude to increases in the amounts of HO enzyme proteins.

The sole study exploring HO enzyme activity in seminal plasma was conducted by ***Abdel Aziz et al. (2008-a)***. They demonstrated positive correlation between seminal plasma HO enzyme activity and sperm concentration, sperm motility percent, motile spermatozoa and sperm normal morphology. They concluded that HO enzyme activity in the human seminal plasma is related to spermatogenesis and sperm-motility processes.

Aim of the Work

This work aimed to assess HO enzyme activity in semen of fertile and infertile males.

Heme Oxygenase

Heme oxygenase (HO-1) catalyzes the oxidative degradation of heme to biliverdin, carbon monoxide, and iron ***(Tenhunen et al., 1969)***. Removing toxic heme is not the only function of HO-1 as its products have been recognized to play important roles in numerous organs. HO-1 is evolutionarily conserved, and it has been detected in bacteria, plants, fungi, and animals ***(Wilks, 2002)*** confirming its importance for the proper function of different organisms.

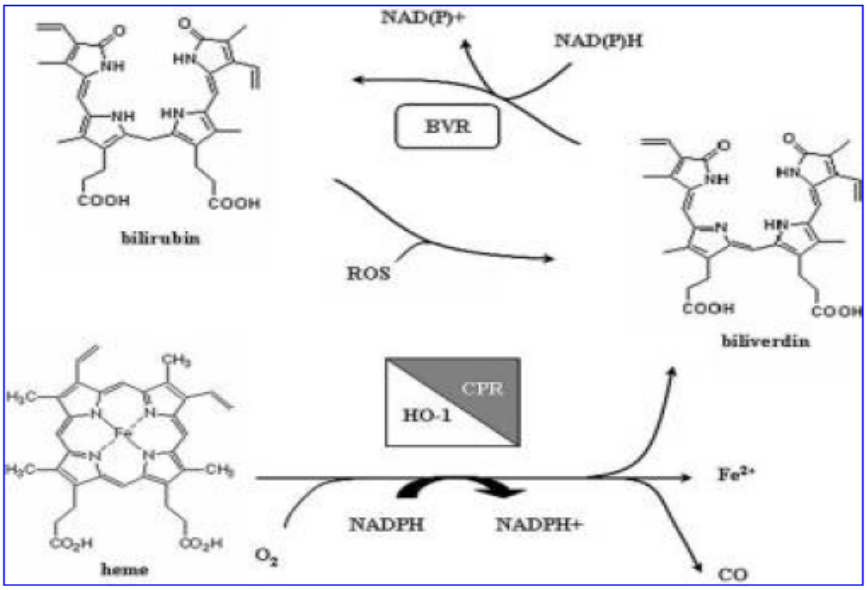
HO: Genes, expression and activity

In the late 1960s, HO was demonstrated to participate, as the first and rate-limiting enzyme, in the conversion of heme into a bile pigment, bilirubin . ***(Tenhunen et al., 1968)***. The sources of heme are hemoglobin and other heme-containing proteins; myoglobin, cytochromes, peroxidases, and respiratory burst enzymes. Heme degradation is energy consuming, and NADPH donates electrons through cytochrome P450 system. Three moles of molecular oxygen (O^2) are consumed for liberating iron from the porphyrin ring of heme, release of carbon monoxide (CO), and formation of biliverdin

(Maines and Kappas 1977; Yoshida and Kikuchi, 2006). Biliverdin is quickly reduced to bilirubin.

A. HO isoforms

Two isoforms of HO have been identified: an inducible form, HO-1, and a constitutive form, HO-2 ***(Maines et al., 1986)***. Moreover, HO-3, a pseudogene derived from an HO-2 transcript ***(McCoubrey et al., 1992)***.



Heme-degradation pathway.

HO, cooperating with NADPH cytochrome P450 reductase (CPR), degrades heme to produce free iron, CO, and biliverdin, which is rapidly converted to bilirubin by BVR. Reactive oxygen species (ROS) can be scavenged by bilirubin, protecting the cell from oxidative stress. ROS oxidizes bilirubin to biliverdin, which can be reduced to bilirubin by BVR, completing a catalytic cycle.

HO-1, a 32-kDa protein also known as the stress protein HSP32, is considered a protective, early stress-response gene, the expression of which is generally not detected in normal tissues, apart from the spleen, where it is

predominant even under normal, unstressed conditions ***(Tenhunen et al., 1969)***. Its low basal expression can be upregulated by a variety of stimuli causing oxidative stress, including heme, cobalt protoporphyrin (CoPP), heavy metals, cytokines, lipopolysaccharide (LPS), hydrogen peroxide (H_2O_2), growth factors, heat shock, ultraviolet light and CO ***(Kim et al., 2007)***.

HO-2, a 36-kDa protein, is present mostly in the brain and at lower levels in testes, endothelium, distal nephron segments, liver, and mysenteric plexus, with subcellular localization in the mitochondria. Its expression is generally constant and can be augmented only by limited factors as dexamethasone or corticosterone. However, depending on the cell type and microenvironment, its expression can be both upregulated and down-regulated by hypoxia ***(Han et al., 2003)***. HO-2 may regulate expression of HO-1 by modulating the cellular heme levels. HO-2 is also involved in calcium signaling and neuroprotection ***(Dore et al., 1999; Boehning et al., 2004)***.

HO-3 gene was initially suggested to encode a 33-kDa protein in different organs but regarded as a pseudogene derived from HO-2 transcript, and it cannot be considered the functional enzyme ***(McCoubrey et al., 1997; Hayashi et al., 2004)***.

Regulation of HO-1 gene

Human HO-1 gene consists of 5 exons and 4 introns, spanning a 14-kb region at human chromosome 22q12. No typical TATA or CAAT boxes are present in the 5'-flanking region of the human gene. However, a TATA-like sequence, ATAAATG, is located 21 bp upstream of the transcription initiation site ***(Shibahara et al., 1989)*.**

1. Inducers and inhibitors of HO-1 expression.

The effect of heme, both as a substrate and an inducer of HO-1 expression, has been widely studied ***(Yoshida et al., 1988; Jazwa et al., 2006; Silva et al., 2006)***. The repression of HO-1 expression by hypoxia, desferrioxamine, or interferon appears to be restricted to human cells ***(Takahashi et al., 1999; Nakayama et al., 2000; Kitamuro et al., 2003)***. Tin and zinc protoporphyrins are widely used inhibitors of HO-1 activity ***(Sardana and Kappas, 1987; Yang et al., 2001; Morioka et al., 2006)***.

Hypoxia and HO-1

Data showed induction of human HO-1 in hypoxic conditions. Moreover, the inhibition of HO-1 observed in human tissues exposed to low oxygen tension was postulated to be an important defense strategy. First, it was hypothesized that the reduced expression of HO-1 by hypoxia may reduce

energy expenditure used for heme catabolism as heme breakdown catalyzed by HO-1 is an energy-consuming reaction ***(Nakayama et al., 2000)***. Also, lower HO-1 expression in hypoxic conditions prevents the local accumulation of CO, iron, and bilirubin.

HO-1 Deficiency

HO-1 deficiency seems to be related to many dangerous side effects, implicating impairment or loss of function of different organs and tissues. Of most important is the injury of vascular endothelium leading to cardiovascular diseases. Apart from the only case of human HO-1 deficiency recognized, it seems that a much more common phenomenon in the human population is variation of HO-1 activity, depending on the length of the GT repeat in HO-1 promoter.

Protective role of HO-1 products

A. Carbon monoxide

Studies as early as in 1991 had discovered that CO may activate the soluble form of guanylyl cyclase (sGC) and be responsible for cGMP formation ***(Furchgott and Jothianandan, 1991)***, thus working in a similar way as NO. Subsequent studies showed its important biologic activities: modulation of vasomotor tone ***(Durante and Schafer, 1998)*** and involvement

in neuronal transmission ***(Dawson and Snyder, 1994)***. Moreover, CO can exert potent anti-inflammatory effects ***(Ryter et al., 2002)***. It was shown to be responsible for the inhibition of pro-inflammatory cytokine production, including tumor necrosis factor (TNF), interleukin-1 (IL-1) and upregulation of IL-10 ***(Otterbein et al., 2000)***.

Another protective CO effect is mediated through GC/cGMP correlated with preventing platelet activation and aggregation ***(Brune and Ullrich, 1987)***. CO also suppresses thrombosis and the pro-inflammatory response stimulated by activated platelets ***(Chlopicki et al., 2006)***. Additionally, in macrophages, CO down-regulates expression of plasminogen activator inhibitor type 1 ***(Fujita et al., 2001)***.

Conversely, CO prevents apoptosis in several cell types, including endothelial cells ***(Otterbein et al., 2003)***, vascular smooth muscle cells ***(Liu et al., 2002)***, fibroblasts ***(Petrache et al., 2000)***, osteoblasts ***(Chae et al., 2006)***, and beta-cells of the pancreas ***(Gunther et al., 2002)***. The protective action of CO in the vascular bed is correlated with its influence on apoptosis of endothelial cells mediated by activation of various signaling pathways. CO is able to modulate Fas/Fas ligand interaction and activation of executors of apoptosis: caspases 3, 8, and 9. The mechanisms of the antiapoptotic effect of

CO involve regulation of the expression of both anti- and proapoptotic proteins ***(Zhang et al., 2003)***.

B. Biliverdin and bilirubin

Biliverdin and bilirubin are known as bile pigments and as waste products of heme degradation. Lately, they have been recognized as cytoprotective agents able to substitute for HO-1 activity. Bilirubin is potentially toxic, but normally it is rapidly conjugated to glucuronic acid for urinary excretion. This bile pigment is a physiologic neuroprotectant, reducing neural damage after various types of hypoxic insults, like stroke and neurodegenerative diseases ***(Dore and Snyder, 1999; Dore et al., 1999)***. Moreover, it protects lipid membranes against oxidation ***(Stocker et al., 1987)***. The description of a cycle in which biliverdin is converted to bilirubin by biliverdin reductase and bilirubin is recycled back to biliverdin on oxidation by peroxyl radicals suggested a mechanism that amplifies the antioxidant effects of the bile pigments. The amplification afforded by this cycle can explain the ability of low-nanomolar concentrations of bilirubin to overcome 10,000-fold higher concentrations of oxidants ***(Baranano et al., 2002)***.

It possesses anti-apoptotic and anti-inflammatory activities. Inhibition of bilirubin production increased the ROS level and promoted apoptotic death

(Baranano et al., 2002). It was shown that slightly elevated circulatory bilirubin reduces risk of atherosclerosis and coronary artery disease ***(Mayer, 2000)***. The potential therapeutic effects of bilirubin for the pharmacologic administration of bile pigments have been suggested. The pharmacologic supplementation with bilirubin protected rats against hepatotoxicity during experimental endotoxemia ***(Wang et al., 2004)*** dependent on decreased inducible NOS (iNOS) activity and NO production, as well as on the diminishment of proinflammatory cytokine expression. Additionally, biliverdin/bilirubin may protect grafts from injury and rejection ***(Fondevila et al., 2003; Yamashita et al., 2004)***.

C. Iron

Iron, a crucial cofactor of many cellular enzymes and redox-dependent proteins, is essential for a number of biologic processes but in higher concentrations it possesses a potential to cause deleterious effects ***(Ong and Halliwell, 2004)***. Iron can be toxic for cells and aggravate oxidative stress ***(Suttner and Dennery, 1999)***. However, free iron up-regulates expression of ferritin, which possesses chelating activity toward iron and confers cytoprotection on endothelial cells ***(Balla et al., 1992)***. Several studies had