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Study of Heme Oxygenase in Placental Tissue of Preeclamptic Patients

Thesis

Submitted in partial fulfillment of Master Degree in Medical Biochemistry

& Molecular Biology

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قَالُوا سُبْحَانَكَ لا عِلْمَ
إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ
الْعَلِيمُ الْحَكِيمُ "

صدق الله العظيم

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INTRODUCTION

Preeclampsia (PE) is one of the leading causes of maternal death. It is characterized by a triad of hypertension, proteinuria, and edema. In this syndrome, there is failure of spiral artery transformation and a reduction in uteroplacental blood flow **(Bower et al, 1993).** PE is associated with widespread maternal endothelial dysfunction, fetal growth restriction (FGR), and evidence of increased oxidative stress within both the maternal and the placental circulations **(Walsh 1998)**.

HO is the rate-limiting enzyme in the heme catabolism. It is a microsomal enzyme that oxidatively cleaves heme, a pro-oxidant, to produce biliverdin, a potent antioxidant, iron, and CO **(Otterbein and Choi 2000).**

HO consists of three homologous isoenzymes:

HO-1, a stress-responsive protein that is expressed at high concentrations in the spleen and liver, where it is responsible for the destruction of heme from red blood cells. It can be induced by various oxidative agents, inflammatory cytokines, heat shock, heavy metals, ultra violet radiation **(Applegate et al., 1991 and Balla et al., 1993)** hypoxia and hyperoxia **(Maines et al., 1993b)**;

HO-2, which is widely distributed throughout the body. HO-2 is thought to be not inducible, however **HO-2** is predominantly expressed in the brain and testes and also constitutively expressed in other tissues, including endothelium, distal nephron segments, liver, mysenteric plexus of the gut, and in other tissues at low levels **(Ewing and Maines 1992).**

HO-3 which might have a regulatory role in heme-dependent cellular processes **(McCoubrey 1997).**

The cytoprotective effects of HO are supported by observations made in the HO-1-deficient [HO-1(–/ –)] mice. The majority of these HO-1(–/ –)mice do not survive to term, and the mice that survive to term were abnormal and died within one year of birth. These adult mice exhibit growth retardation and normochromic, microcytic anemia. Kidneys and livers from these mice show evidence of iron deposition, and as these HO-1(–/ –) mice age, they also demonstrate an increased presence of chronic inflammation characterized by hepatosplenomegaly, leukocytosis, glomerulonephritis, and hepatic periportal inflammation (**Poss and Tonegawa 1997)**. The first identified case of a HO-1-deficient human patient exhibited similar phenotypic alterations as those observed in the HO-1(–/ –) mouse, including growth retardation, anemia, leukocytosis, and increased sensitivity to oxidant stress (**Otterbein and Choi 2000).**

The released free iron (its two free electrons capable of generating the vicious hydroxyl radical through Fenton chemistry with the superoxide radical) is rapidly sequestered into the ***iron storage protein ferritin***. **Eisenstein et al (1991)** clearly showed that ferritin is increased in tandem with HO-1 and decreased with inhibition in HO-1. Over expression of HO-1 also up regulates and interacts with an ***iron ATPase*** present in the endoplasmic reticulum. This iron pump is thought to limit intracellular iron content once HO-1 activity is up regulated. The ability of cells expressing HO-1 to decrease iron content has recently been suggested to account in large measure for the antiapoptotic effects of HO-1 **(Otterbein and Choi 2000)**.

Not only does HO rid the body of free heme, which is very toxic, causing tissue inflammation and injuries, but it also produces biliverdin that is converted to bilirubin, both products being potent antioxidants **(Sollwedel et al., 2005).**

Yoshiki et al., (2000a) reported that **HO-1** is expressed by epithelial cells and macrophages, and **HO-2** in vascular endothelial cells and smooth muscle cells. Recently the distribution of the two HO isoforms in human placental villi had distinct topographic patterns**.**

Sollwedel et al (2005) reported that induction of HO-1, in mice, by Co-protoporphyrin treatment, during implantation, prevented fetal rejection, whereas the down-regulation of HOs by zinc-protoporphyrin application boosted abortion.

Ahmed (2011) reported that chorionic villous sampling from women at eleven weeks gestation shows that HO-1 mRNA expression is decreased in women who go on to develop preeclampsia. These findings provide strong evidence for a protective role of HO-1 in pregnancy and identify HO as a target for the treatment of preeclampsia. In contrast **Eide et al (2008)** reported increased decidual expression and maternal serum levels of HO-1 in PE patients as compared to control pregnant females.

Another cytoprotective product of HO is CO. which, like NO, activates soluble guanylate cyclase to produce cGMP (cyclic guanosine monophosphate) **(Maines 1993).** CO acts as a neurotransmitter **(Verma et al., 1993),** inhibits platelet aggregation **(Mansouri and Perry 1982),** and is a vascular smooth muscle relaxant **(McFaul and McGrath 1987).**

There is increasing evidence that CO is required for normal placental development and function; endogenous CO production may regulate trophoblast migration and organization (**Roberts**

and Lain 2002 and Bainbridge et al., 2005) and play a hemodynamic role **(Bainbridge et al., 2002)**. In placental perfusion studies, inhibition of HO activity led to a dose-dependent increase in perfusion pressure, suggesting that CO may also operate as a vasodilator in the placenta **(Lyall et al., 2000).** Women who have PE have significantly decreased CO concentrations in their exhaled breath compared with healthy pregnant women **(Kreiser et al., 2004).** Furthermore, a reduced risk for PE is found in women smoking cigarettes throughout pregnancy. The reduction in risk is 33% compared to nonsmoking women **(Bainbridge et al., 2005)** This was ascribed to exposure to elevated concentrations of exogenous carbon monoxide (CO), one of the combustible by-products of cigarette smoke.

Aim of the work:

Considering the cytoprotective antioxidant, anti inflammatory and antiapoptotic role of HO and its products, this study is carried out to evaluate the extent of HO expression in placenta of preeclamptic patient in comparison to that of normal pregnancy placenta.

The results will be correlated with the different parameters defining pregnancy outcome.

Hemeoxygenase

Hemeoxygenase (HO) is the enzyme that catalyzes the first and rate-limiting step in the degradation of heme. It was originally identified by **(Tenhunen et al., 1969)**, where they characterized HO enzyme as well as its cellular localization.

HO is classified according to enzyme classification as EC 1.14.99.3, its systematic name is heme, hydrogen-donor: oxygen oxidoreductase (α-methene-oxidizing, hydroxylating), other names also includes heme oxidase.

HO catalyzes the following reaction

Heme + 3 AH_2 + 3 O_2 = Biliverdin + Fe^{2+} + CO + 3 A + 3 H_2O

AH_2 is a reduced substrate and

A is its oxidized form.

(Yoshida et al., 1974, Maines et al., 1977, and Sunderman et al., 1982).

HO isoforms

Heme-oxgenase has three isoforms (HO-1, HO-2, and HO-3) that catalyze this reaction; they have been identified by (**Abraham et al., 1987, Maines 1988, and McCoubrey et al., 1997).**

HO-1

Is a 32-kDa protein that is inducible by numerous stimuli **(McCoubrey et al., 1997).**The human HO-1 gene is located on chromosome 22q12 (**Abraham et al., 1996)**, and consists of 5 exons and 4 introns, spanning ~14 kb **(Fogg et al., 1999).**

Distribution of Heme Oxygenase 1

HO-1 is most abundantly expressed in the spleen. In fact, under physiological conditions, the spleen may be the only organ in which HO-1 overpowers HO-2, but the HO isoforms expression can change under different conditions. Upon stimulation, HO-1 expression in the testes overpowers the expression of HO-2. The same is true for the lung, brain, and other tissues **(Ewing and Maines, 1992)**, recent studies have detected the HO-1 mRNA in different regions of the brain,

especially in the hippocampus and the cerebellum **(Scapagnini et al., 2002)**.

Subcellular localization of HO-1

HO-1 is traditionally viewed as a microsomal protein, primarily localized in endoplasmic reticulum **(Maines, 1988).** HO-1 has also been detected in cytoplasm, nuclear matrix, mitochondria, and peroxisomes of parenchymal and nonparenchymal liver cell populations by monitoring the formation of bilirubin as an indicator of HO activity **(Srivastava and Pandey 1996)**. This protein can be translocated to the nucleus of differentiated cells where it may participate in the regulation of heme metabolism. The translocation of HO-1 to the nucleus would be a factor in the regulation of different transcriptional factors **(LiVolti et al., 2004).**

Regulation of HO-1 expression

(A) Up-Regulation of Heme Oxygenase 1

HO-1 is also known as the stress protein HSP32 **(Keyse and Tyrrell 1989)**. A large number of studies deals with the regulation of HO-1 gene expression by stimuli that increase the cellular production of ROS (reactive oxygene species) such as heme, heme derivatives, heavy metals, UV light, hydrogen

peroxide, lipopolysaccharide (LPS), or by stimuli that deplete cellular glutathione stores **(Applegate et al., 1991; Choi and Alam 1996).** Furthermore, it has been shown that scavengers of ROS inhibit and reduce the magnitude of HO-1 induction by oxidative stress **(Lautier et al., 1992).** These findings indicate that an increase of intracellular ROS and, therefore, the activation of redox-dependent signaling pathways play a crucial role for the regulation of HO-1 gene expression **(Finkel 1998).** Another great array of endogenous and exogenous stimuli can also induce the expression of HO-1as Nitric oxide, Cytokines, Angiogenesis, as well as Endotoxin, Hypoxia, Heat shock, Prostaglandins, Phorbol esters, Sodium arsenate **(Otterbein and Choi 2000).**

(B) Down regulation of HO-1

In contrast to the up-regulation of HO-1 gene expression, much less is known about the regulatory mechanisms that suppress HO-1 gene expression. The reduced expression of HO-1 seems to play a role in preserving intracellular heme as an important substrate of certain heme proteins, and reduce energy expenditure for heme catabolism. The repression of HO-1 by hypoxia or heat shock is unique for many types of human cells such as human retinal pigment epithelium cells **(Udono-Fujimori et al., 2004),** and the same hypoxia stimuli increase the expression of HO-1 in rodent cells **(Shibahara et al., 2003).**
HO-1 gene expression can be down-regulated by angiotensin II in rat vascular smooth muscle cells which is apparently mediated by an increase of intracellular calcium levels **(Ishizaka and Griendling 1997).** Moreover; it has been shown that interferon-γ suppresses HO-1 gene expression in human glioblastoma cells **(Takahashi et al., 1999).** Bach1, a member of basic leucine-zipper factors, is reported as a heme-regulated transcriptional repressor for the HO-1 gene **(Sun et al., 2002).**

Depending on the cell types and the nature of the stimuli, HO-1 induction may be mediated by different signaling pathways. Stimulation of the HO-1 gene by most, if not all, stimuli is primarily controlled at the Transcriptional level **(Choi** and **Alam**