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REVIEW ARTICLE

Heme Oxygenase-1(HO-1) as a Potential Target for Cytoprotection- A Review

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ABSTRACT

Cytoprotection i.e. protection of cells from harmful agents, physiologic stress and pathologic stimuli is and has always been of prime importance. Heme oxygenase (HO) is an enzyme that can help modulate and protect cellular life. HO is a significant rate-limiting enzyme that metabolizes heme to produce equimolar amounts of Biliverdin, Carbon monoxide (CO) & free iron. In this action, the activity of NADPH-Cytochrome P-450 reductase is required. Biliverdin is then reduced by biliverdin reductase to Bilirubin which protects the cells from oxidative stress by scavenging oxygen free radicals. CO stimulates soluble Guanylate Cyclase (sGC) leading to increased production of cGMP & alters smooth muscle cell activity causing vasodilation. The free iron is sequestered into Ferritin which, when HO expression is elevated, plays a vital role in removing Fe^{2+} from cell. Three isoforms of HO have been identified out of which HO-1 is inducible and HO-2 is constitutively expressed. HO-3 is a pseudogene. Upregulation of HO-1 is controlled by upstream signaling kinases namely extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK (Mitogen-activated protein kinase). Various Transcription factors too have a role in activating the HO-1 gene. HO-1 plays a protective role in many disease states such as Atherosclerosis, Alzheimer's disease, Parkinson's disease, Hepatitis etc. The focus of this review is the significance of targeted induction of HO-1 as a potential therapeutic strategy to protect cells from harmful agents.

KEYWORDS

Cytoprotection, Heme Oxygenase-1, Oxidative Stress, Signaling Kinase, Transcription Factors

INTRODUCTION

Cells undergo stress and are affected by noxious stimuli but they adapt and preserve their function as they accommodate the changes by maintaining homoeostasis. But if the external stress or the noxious stimuli is exceedingly harmful or if the cells are exposed to damaging agents then it leads to cell injury. If the cell injury is irreversible then the affected cells die. Cell death disrupts the tissue and has been implicated in a number of pathological conditions. Inability to regulate cell death are a part of many disease conditions such as AIDS, cancer, ischemia, liver diseases and neurodegenerative disorders such as Parkinson's and Alzheimer's disease. Cytoprotection i.e. protecting the cells from harmful agents, physiologic stress and pathologic stimuli has always been a topic of interest among budding researchers and a lot of successful strategies have been devised for achieving it[1,2]. Here in this review we focus on the significance of targeted induction of HO-1 as a potential therapeutic strategy to achieve cytoprotection.

Heme is a complex of a metal iron chelated in a porphyrin ring. Heme is synthesized partly in the mitochondria and partly in the cytoplasm.

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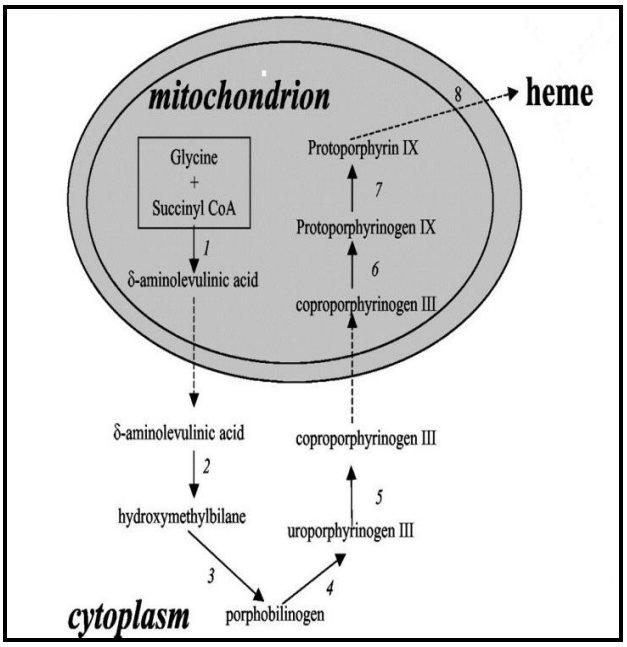

Figure 1: Overview of Heme Synthesis

Heme is synthesized in all human nucleated cells. Heme is incorporated in large quantities into the heme proteins, hemoglobin and cytochrome P450 of the erythroid cells in the bone marrow. Free heme can be toxic to cells as leads to the production of reactive oxygen species. The amount of heme needs to be controlled in order to avoid pathological conditions[3]. This is where heme oxygenase comes into picture as its main function is to cause heme degradation.

Heme oxygenase (HO) is a significant rate limiting enzyme in the metabolism of heme to yield equimolar amounts of biliverdin, carbon monoxide (CO) and free iron. Heme oxygenases are located within the endoplasmic reticulum, where they serve, in co–ncert with NADPH-Cytochrome P-450 reductase, to oxidize heme to Biliverdin, CO and free ferrous iron. The heme ring is cleaved at the α-methene bridge by HO to form biliverdin. Biliverdin reductase then reduces the γ-methene bridge of Biliverdin to form Bilirubin[4,5,6].

Bilirubin acts as a potent antioxidant by protecting the cells from oxidative stress as it scavenges oxygen free radicals. CO stimulates soluble Guanylate Cyclase (sGC) leading to increased production of cGMP & alters smooth muscle cell activity causing vasodilation. The free iron is sequestered into Ferritin which, when HO expression is elevated, plays a vital role in removing Fe^{2+} from cell.

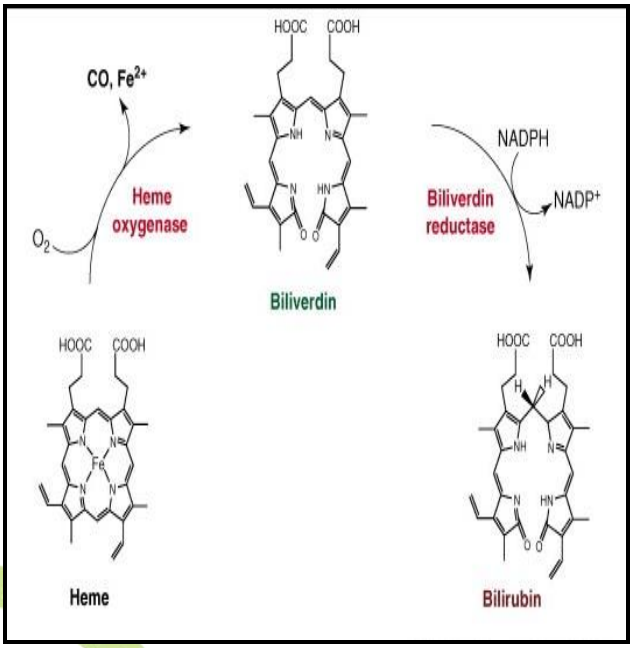

Figure 2: The Heme Degradation Pathway: Heme is enzymatically degraded to yield free iron, CO & Biliverdin-which is subsequently converted to bilirubin by biliverdin reductase).

Out of the 3 isoforms of HO [HO-1, HO-2 & HO-3], HO-3 is a pseudogene. HO-1 is also known as heat shock protein 32 as it is a 32kDa protein. It is an inducible protein known to be located in endoplasmic reticulum, mitochondria, nuclei and caveola. It is ubiquitously present in mammalian tissues such as liver, spleen, pancreas, intestine, kidney, heart, retina, prostate, lung, skin, brain, spinal cord, vascular smooth muscle cells and endothelial cells. The HO-1 gene, in a human, is approximately 14kb long and contains five exons. HO-1 is activated by a variety of inducers that includes heme, heavy metals, hydrogen peroxide, oxidized low density lipoproteins, hyperoxia, hypoxia, endotoxins, nitric oxide, cytokines, shear stress, ultraviolet radiation and any oxidative stress inducers. Under physiologic conditions, its expression is low except in spleen where the action of HO-1 is important for recycling of iron from old and damaged erythrocytes. When there is acute induction and expression of HO-1 then its reaction products act as a cytoprotective.

However if the induction of HO-1 is chronic then it leads to depletion of heme and subsequently loss of heme required for normal cellular function. HO-2 is constitutively expressed i.e. it is virtually uninducible but is present chiefly present in brain and testes. Both HO-1 and HO-2 cause heme degradation. Deficiency of HO-1 in humans is associated with susceptibility to oxidative stress and increase in pro-inflammatory state which leads to endothelial damage[7,8,9,10]. The protective effects of HO-1 have been studied in a variety of models for inflammation, apoptosis etc.

Induction & Regulation of HO-1 Gene Expression

HO-1 is known to be induced by a wide array of stimuli including heme, heavy metals, hydrogen peroxide, endotoxins, hypoxia, hyperoxia, haemorrage, cytokines, ischemia-reperfusion, halothane etc. The most important function of HO-1 is to make sure that the heme levels are regulated at all times. Acute induction of HO-1 has a beneficial effect because of rapid decrease in undesired heme. Chronic induction of HO-1 can have detrimental effects in cellular metabolism as there will be depletion of heme and subsequently loss of cellular heme proteins required for normal cellular function[11,12].

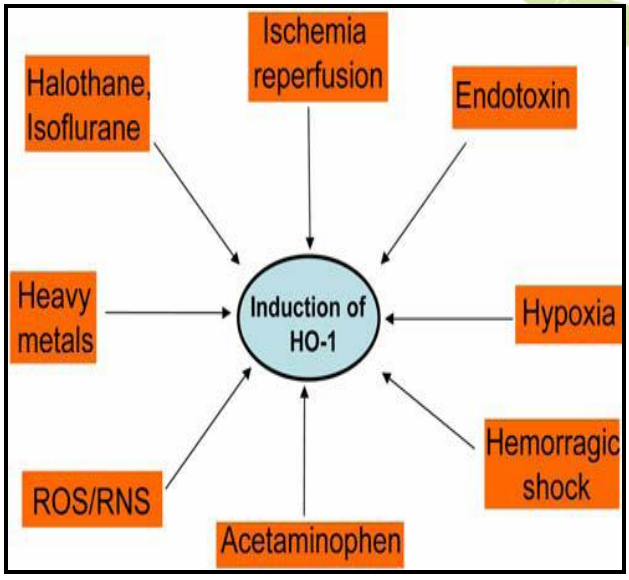

Figure 3: Induction of HO-1 by a variety of stimuli

To regulate the induction of HO-1 many intracellular signaling molecules are involved. Upstream signaling kinases i.e. extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK (Mitogen-activated protein kinase) play vital roles in controlling up-regulation of HO-1. Other signaling enzymes known to be involved in HO-1 induction include protein kinase (PKC), protein kinase A (PKA) and phosphatidyl inositol 3-kinase (PI3K). Intracellular levels of cAMP are elevated by a large number of hormones and external stimuli, resulting in the activation of PKA. cGMP formed via activation of soluble guanylate cyclase, either directly by NO-releasing agents or by the induction of of inducible nitric oxide synthase (iNOS), upregulates HO-1 gene expression. The PI3K/Akt pathway controls the intracellular levels of ROS by regulating the expression of HO-1. Activator protein (AP)-1 is a major transcription factor that can transactivate HO-1 by binding to the promoter region of the gene. Nrf2 (Nuclear factor 2), another transcription factor, is sequestered in the cytoplasm as an inactive complex with its cytosolic repressor Keap1.

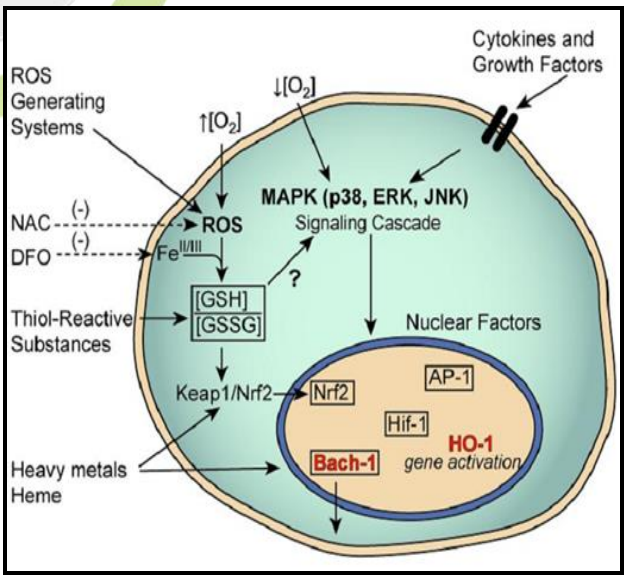

Figure 4: Regulation of HO-1 by Transcription Factors and their Upstream Signaling Kinases - extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK (Mitogen-activated protein kinase).

Under normal conditions, transcription factors, such as NF-κB (Nuclear Factor- 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. Increases of intracellular reactive oxygen species lead to perturbation of intracellular thiol equilibrium, leading to reduction of GSH (reduced glutathione)/GSSG (oxidized glutathione) ratio, and redox regulation of Keap1. Treatment with heavy metals and/or heme promotes Nrf2 nuclear translocation and nuclear export of the transcriptional repressor Bach1. 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), leading to the expression of HO-1. Antioxidants N-acetylcysteine (NAC) and metal chelators such as DFO (Desferrioxamine) inhibit HO-1 activation by preserving thiol status or removing iron (Fe) involved in the amplification of ROS-mediated events[13,14].

Cytoprotective Role of HO-1

Cytoprotection is a process by which protection is provided to cells against harmful agents. General mechanism of cytoprotection includes improvement in membrane fluidity, modification of cytokine expression, reduction of free radicals, modulation of apoptosis & stabilization of mitochondrial function. The products of HO-1 reaction i.e. Biliverdin & Bilirubin, CO and free iron play a vital role in achieving cytoprotection. By achieving cytoprotection we can protect tissues from various diseases. We discuss below how the products of heme degradation via HO confer cytoprotection and the mechanism by which cytoprotection is achieved.

Biliverdin and Bilirubin

Both Biliverdin and Bilirubin possess antioxidant properties. They are reducing agents and protect the cells from oxidative stress by scavenging oxygen free radicals and thus prevents uncontrolled formation of Reactive oxygen species (ROS).

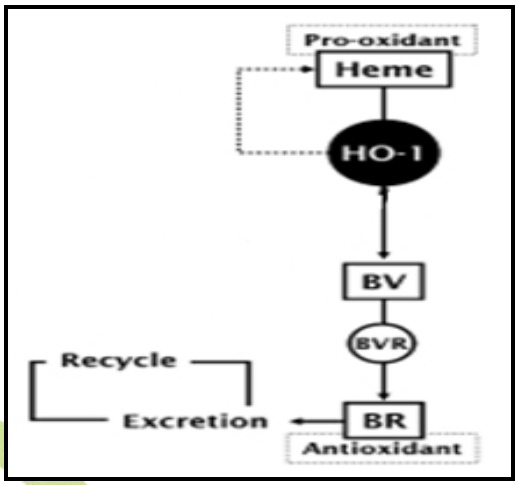

Figure 5: Biliverdin (BV), which is converted to Bilirubin (BR), by Biliverdin reductase (BVR) acts as an Anti-oxidant and confers Cytoprotection

Higher concentrations of Bilirubin is toxic. Therefore regulation of HO-1 induction and expression is crucial. Bilirubin can easily cross biological membranes compared to Biliverdin as Bilirubin is more lipophilic. Conjugation of Bilirubin mainly occurs in the liver whereas Bilirubin produced in other organs is transported in plasma mainly bound to albumin. Bilirubin inhibits NADPH oxidase and PKC activity. Both enzymes have been shown to mediate angiotensin II-induced vascular injury. Also biliverdin and bilirubin have been shown to preserve endothelial cell integrity and prevent endothelial cell death[15].

Carbon Monoxide (CO)

HO-1 is the most important source of endogenous CO production. CO stimulates soluble Guanylate Cyclase (sGC) leading to increased production of cGMP & alters smooth muscle cell activity causing vasodilation. Also CO produces vasorelaxation by stimulating K

channels, increases angiogenesis, and is antiapoptotic and anti-inflammatory.

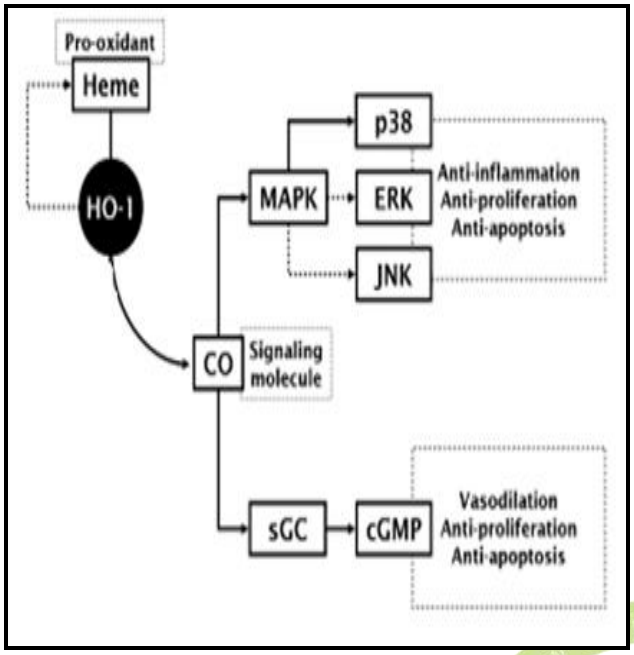

Figure 6: CO and its mechanism by which it confers Cytoprotection

CO suppresses the pro-inflammatory response while it enhances the anti-inflammatory function of macrophages. CO inhibits platelet activation and aggregation, thereby suppressing thrombosis and the pro-inflammatory damage induced by activated platelets. Other anti-inflammatory effects triggered by CO include down regulation of the expression in macrophages of plasminogen activator inhibitor and prevention of apoptosis in several types of cells, such as endothelial cells, fibroblasts and hepatocytes. CO can cause a down regulation of proinflammatory cytokine production through p38 MAPK-dependent pathways leading to anti-inflammatory tissue response[16,17,18,19,20,21].

Free Iron and Ferritin

Metabolism of heme leads to the production of iron. Even when present in low concentration iron is known to cause cytotoxicity by catalyzing the production of hydroxyl radicals. Iron generates ROS and damages cellular components. Ferritin is a protein that sequesters iron and it is elevated when HO-1 is induced and decreased when the activity of HO-1 is suppressed. The ATPase pump that actively removes intracellular iron from the cell is increased with the expression of ferritin, and in this process the intracellular pool of iron is decreased. Both ATPase pump and ferritin play vital roles in removing iron from the cell when expression of HO-1 is elevated. Thus Ferritin acts as an antioxidant.

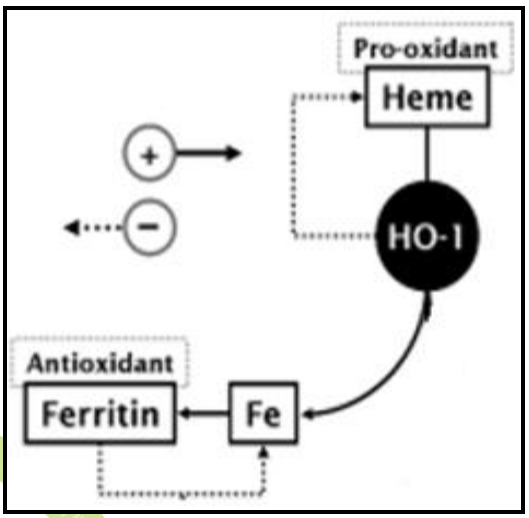

Figure 7: Ferritin removes free iron and serves as a Cytoprotective antioxidant

Ferritin has been known to protect endothelial cells from oxidized LDL and iron induced oxidative stress.

Therapeutic Role of HO-1

Oxidative Stress and Inflammation are known to be an important constituent and pathogenesis of most of the diseases such as Atherosclerosis, Alzheimer's disease, Parkinson's disease, Hepatitis, Cancer, Heart failure etc. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Examples include oxygen ions and peroxides. Reactive nitrogen species (RNS) are a family of antimicrobial molecules derived from nitric oxide and superoxide produced via the enzymatic activity of inducible nitric oxide synthase 2 (NOS2) and NADPH oxidase respectively. When there is an imbalance in the levels of ROS and RNS then the damage caused to the cells by the is extreme to such levels that it becomes difficult for the biological system to repair the damage. This leads to oxidative stress which is the result of excessive production of oxidant species and/or depletion of intracellular antioxidant defences,

leading to an imbalance in the redox status of the cell. Oxidative stress triggers necrosis and apoptosis. In response to pro-oxidant stimuli, such as cytokines or β-amyloid, free radicals(FR) which include ROS, nitric oxide (NO) and RNS, induce the expression and activity of both heat shock protein 70 (Hsp70) and heme oxygenase-1 (HO-1). Hsp70 inhibits caspases and NFkB, while activating members of the MAPK family, such as ERKs and p38. Conversely, HO-1 converts heme into carbon monoxide (CO) and biliverdin (BV) which is then reduced by biliverdin reductase (BVR) into bilirubin (BR). BR is a well known scavenger for FR thus allowing a negative feedback to prevent uncontrolled formation of ROS, NO and RNS. Hsp70 induces HO-1, thus potentiating cytoprotective action (Figure 8). The byproducts of HO-1 mediate antioxidant and anti-inflammatory activity by upregulating Nrf2 activation and ferritin expression and downregulating NF-κB. The anti-inflammatory cytokine IL-10 (interlukin-10) can induce the expression of HO-1[22,23,24,25,26].

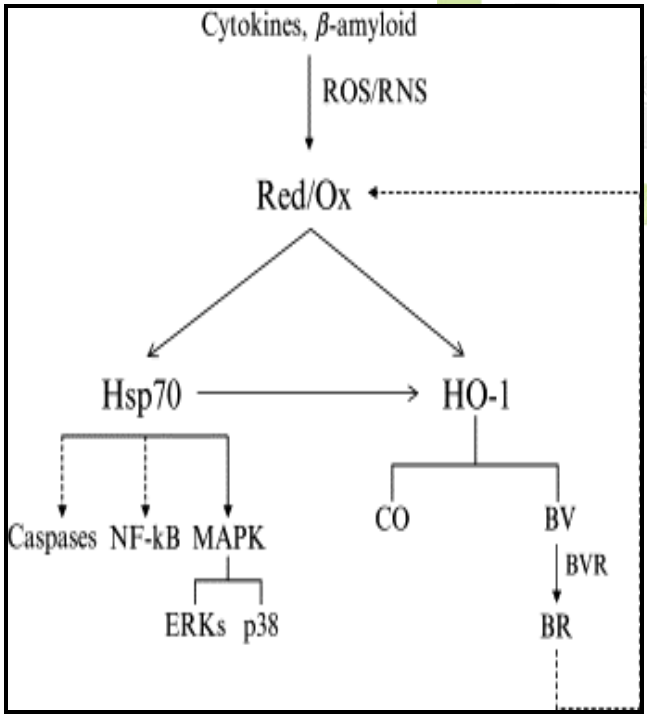

Figure 8: Free radicals induce expression of Hsp 70(Heat shock protein 70) and HO-1 and confer Cytoprotection

Ischemia and Reperfusion are one of the major causes for organ failure and cell injury. Ischemia or lack of oxygen occurs when blood supply to a particular area of tissue is cut off. Reperfusion is the restoration of blood supply after ischemia. But the lack of oxygen and nutrients during ischemia causes inflammation and Oxidative stress and it increases after restoration of blood. This increase is due to the activity of free radicals. HO-1 is known to protect the tissues like heart, lung, liver, kidney and brain from ischemia and reperfusion injury. One possible mechanism for this cytoprotection is by the modulation of the pro- and anti-apoptotic pathways by HO-1.

Both CO and bilirubin play a role in mediating the protective effects of HO-1 expression in ischemia-reperfusion injury.

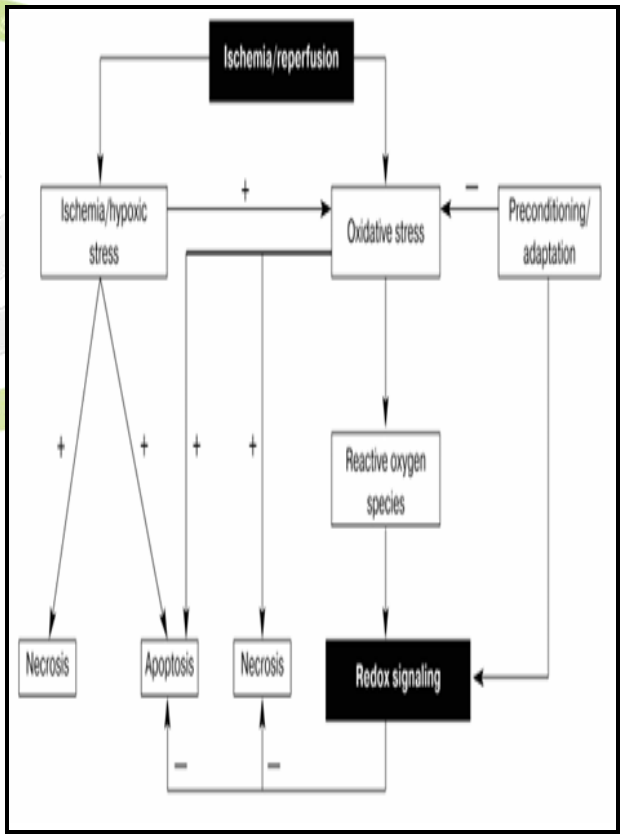

Figure 9: Oxidative stress plays a major role in Ischemia-Reperfusion injury

Thus, HO-1 expression serves as a protective shield in ischemia-reperfusion injury, the effects of which are mediated via CO and/or bilirubin[27,28,29,30].

Hypoxia is a pathological condition in which the body as a whole or a region of the body is deprived of adequate oxygen supply. Chronic hypoxia induces expression of heme oxygenase-1 (HO-1). Induction of HO-1 during chronic hypoxia is necessary for the continued breakdown of heme for the enhanced production of hemoglobin and the increased respiratory and sympathetic responses. Nitric oxide (NO), an end-product of the nitric oxide synthase (NOS) reaction, inhibits heme oxygenase. Carbon monoxide (CO), an end-product of the HO reactions, inhibits NOS. The cytoprotective enzyme HO-1 may be expressed by potential inducers through the mechanisms involving MAPK-dependent activation of Nrf2, and HO-1 catalyzes heme degradation to BV/BR, CO, and iron.

Figure 8: During Chronic Hypoxia, there is an initial increase in NOS and NO, with a subsequent decrease in HO-2 and an increase in HO-1.

These by-products mediate the antioxidant/anti-inflammatory and anti-allergic properties of HO-1, by up-regulating Nrf2 activation and ferritin expression and down-regulating NF-κB[31,32,33].

Future Prospects

There will be a lot of strategies that can be applied by the researchers in the future that will pave the way to combat diseases. Cardiac-specific expression of HO-1 reduces oxidative stress and cell death in the heart following myocardial Ischemia-Reperfusion. The effects of HO-1 are mainly mediated by the production of carbon monoxide (CO) and bilirubin. In the future, it may be possible to utilize a single administration of a drug using site-specific expression of HO-1/HO-2 to promote long-term prophylaxis and to promote myocardial repair in those at high risk of myocardial injury.

Gene therapy i.e. introduction of ho-1 gene into tissues can be one of the approaches to achieve cytoprotection but care should be taken to minimize overexpression of HO-1 gene.

CONCLUSION

The HO enzyme system has undergone interesting transformations, from the study of a key biochemical reaction mechanism involved in the degradation of heme to an important stress response molecule critical in maintaining cellular and tissue homeostasis. The metabolic products of HO-catalysed heme breakdown products have been shown to play pivotal role in achieving cytoprotection. Biliverdin, Bilirubin, CO and ferritin formed by HO reaction, play critical tissue-protective roles against oxidative and ischemic tissue injuries. 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. The inducible enzyme HO-1 is protective for tissue injury through multiple mechanisms including anti-oxidation, anti-inflammation and anti-apoptosis. Induction of HO-1 occurs as an adaptive and beneficial response to several injurious stimuli including heme and this inducible nature of HO-1 signifies its importance in several pathophysiological disease states. Upregulation of HO-1 by various stimuli also modulates key biological processes including inflammation and ischemic injury. HO-1 has thus emerged as a key target molecule with therapeutic implications that elicits cytoprotective response.

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