ROLE OF CARBON MONOXIDE/ HEME-OXYGENASE PATHWAY IN DIABETIC NEUROPATHIC PAIN

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Abbreviations: HO- heme oxygenase, CO- carbon monoxide, IRP- iron regulatory protein, VSMC- vascular smooth muscle cells, MCI- mild cognitive impairment.

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ABSTRACT
Carbon monoxide known to be one of the environmental pollutant is now believed to play an crucial role in cellular functions like vasodilation and circardian rhythm. Heme Oxygenase is an enzyme which is responsible for CO production. This review will cover the effect of CO in periphery, also its toxicity. Then relation of CO with MAPK pathway will be discussed through which the protective role is exhibited. Finally, this review will throw a light on role of CO in various neurological diseases like multiple sclerosis and also in neuropathic pain. In the end, we discuss the role of Heme oxygenase in different neurological disorders like PD and AD.

Please cite this article in press as Ankit Dhir et al. Role of carbon monoxide/heme-oxygenase pathway In diabetic neuropathic pain. Indo American Journal of Pharm Research.2013:3(7).
INTRODUCTION

Heme oxygenase (HO) catalyzes the conversion of heme to biliverdin, iron, and carbon monoxide. Recent studies conducted on different pain models have proven the role of HO inhibitors as an analgesic. When oxidative catabolism of Heme occurs due to action of HO enzyme, Co is generated, which acts as a second messenger which further plays a role in biological system. In biological system, two forms of HO are found; inducible(HO-1) and constitutive(HO-2 and HO-3). Inducible forms regulated by multiple stress stimuli. Moreover, this inducible form has shown to be exhibiting protection in, in vitro and in vivo against oxidative stress. HO exerts anti-oxidative functions by converting heme, whose intercellular accumulation may elevate intracellular pro-oxidant status [1], into the bile pigments, biliverdin-IXα, and bilirubin-IXα, which have potent antioxidant properties [2]. The reactive iron released from heme by HO activity may follow detoxification pathways involving either sequestration or extracellular efflux [3,4,5]. Inactivation of iron regulatory protein (IRP) activity, stimulates the formation of the iron confiscation protein [6,7], promoting a secondary cellular desensitization to oxidative stress [8,9]. Thus (I) describe the regulation of HO-1 as an inducible source of endogenous CO, (II) describe evidence that HO-1 acts as a mediator of cellular and tissue protection against oxidative stress, and (III) emphasize recent studies that introduce novel anti-apoptotic and anti-inflammatory properties of HO-derived CO in oxidative lung injury models. The mechanisms by which HO-1 shows anti-inflammatory actions are not understood clearly. When pro inflammatory free heme is degraded enzymatically into biliverdin, CO and free iron, which may plays a major roles to counteract inflammatory reactions. Heme acts as a prosthetic group of haemoglobin, myoglobin and cytochromes and physiologically play important role for mitochondrial electron transport. On the other hand, non-protein bound free heme is highly toxic as it may cause oxidative stress. Also, free heme has also been shown to have proinflammatory properties.

Biliverdin has been shown to be a beneficial compound with potent antioxidant and anti-inflammatory effect [10]. HO-1 acts as an inducible mediator of cellular and systemic defence against oxidative stress, in models of inflammation, ischemia-reperfusion, hypoxia, and hyperoxia mediated injury. In recent studies, heme oxygenase 1 (HO-1) has emerged as an important mediator of antioxidant and tissue protective actions. HO-1 knockout studies has confirmed the important role of this enzyme in cellular anti-oxidant defence and also in vascular protection. Besides vasculature HO-1 shows its anti- inflammatory and cytoprotective action in tissues like heart, kidney and neuronal cells [11, 12]. Among the HO inducers in the endothelium, NO and atrial natriuretic peptide, activates CGMP pathway and induces HO-1 enzyme, and this prevents oxidative injury [11,12].

HO-1 shows its anti-inflammatory responses via metabolic conversion of pro inflammatory free heme into biliverdin and CO. CO is mobilized for signaling, apart from two intuitive mechanisms (I) the availability of substrate heme for enzymatic degradation, and (II) the availability of active HO enzymes, a process which in turn may be regulated by the transcriptional activation of the ho-1 gene by stress, and the possible modulation of ho-2 by glucocorticoids [13]. Physiological roles for CO, which directly involve modulation of cGMP levels, include neurotransmission, vasodilation, the inhibition of platelet aggregation, and anti-proliferative effects on smooth muscle [13,14,15,16,17]. In VSMC, an elevation of cGMP occurred following exogenous CO treatment [15].CO derived from VSMC had paracrine effects on endothelial cells in co-culture, stimulating the production of endothelial cGMP, and suppressing the expression of endothelial-derived mitogen (PDGF, endothelin 1) [15]. Both exogenously applied CO, or hypoxia induced CO had ant proliferative effects on VSMC, associated with elevation of cGMP, and inhibition of transcription factor E2F, a regulator of cell cycle control [16]. During hypoxia, increased CGMP is associated with HO-1 elevation; this could be inhibited by SnPP and the CO scavenger haemoglobin, but not inhibitors of NOS [15].
BIOLOGY AND PHARMACOLOGY OF HO/CO AND THEIR MODULATORS

Carbon monoxide is a low molecular weight diatomic gas that occurs ubiquitously in nature as an air pollutant. Environmental CO arises from the oxidation or combustion of organic matter (i.e. wood, coal, gasoline, natural gas, tobacco). In man, endogenous CO arises principally from heme degradation (>86%). The remainder arises from other sources that may include lipid peroxidation, and xenobiotic metabolism. CO-a toxic gas, now been believed to play a major physiological function as signalling molecule [18,19]. CO, which is derived from HO-1 is involved in the regulation of inflammation, apoptosis and vasodilation. According to one earlier report, when exogenous was administered, it blocked LPS induced production of pro inflammatory cytokines by modulating p38 MAP kinase [11]. CO increases the production of cGMP is further causes vasodilation and blockage of smooth muscle cell proliferation as occur in other signalling gases like NO. CO releasing molecules (CORM’s) are the compounds that release CO to its target sites without the toxicity as seen with gaseous CO may form major future potential in therapeutic applications. HO enzyme is responsible for endogenous production of CO and this enzyme is highly expressed in many inflammatory diseases and thus plays a protective role.

In inflammatory pain models P2X4 receptors are up-regulated and this receptors knocked out models have shown reduced pain behaviours. HO-1 being an antinociceptive, and it was investigated that can P2X4 be a target for CO and tricarbonyldichlororuthenium (II) dimer (CORM-2). P2X4 receptors are unlikely to show antinociceptive effects of HO-1 as these receptors are not the targets for endogenously produced CO. CORM-2 is an effective antagonist at human P2X4 receptors in inflammatory pain models. The role of P2X4 receptors is not well understood. There are several reasons for this, which include (a) their fast recycling to the plasma membrane, the physiological significance of which is not well understood; (b) when the membrane is disrupted, as during whole-cell recordings, membrane currents appear to run down due to an unknown mechanism [20] and (c) a lack of potent antagonists and other pharmacological tools for discriminating P2X4 receptors. Currently, ivermectin is the primary tool for positive discrimination of P2X4 receptors. Ivermectin is an allosteric modulator of P2X4 currents, potentiating the peak current and increasing the duration of currents evoked by brief ATP applications showed that P2X4 was up-regulated in spinal cord microglia following L5 nerve ligation injury, an established model of neuropathic pain [21,22,23]. Similarly, also showed that P2X4 was up-regulated in spinal cord microglia following hind-paw formalin injection, an established model of inflammatory pain [24]. Hydrolysis of heme by HO enzyme is an oxygen dependant process which results in endogenous production of Carbon monoxide (CO). CO is now known as an important signalling molecule in physiological and path physiological processes and has been shown to be responsible for many of the protective effects observed when HO-1 is upregulated following cellular stress [18]. Increased HO-1 expression induced by cobalt protoporphyrin or epibatidine intraperitoneal injection causes a reduction in nociceptive behaviours in the formalin injection inflammatory pain model [25,26]. Injection of heme oxygenase inhibitors, or minimising HO-1 upregulation with a dominant negative Nrf2 transcription factor, negates the protective effect of HO-1 upregulation [25,26]. There are many proposed cellular targets of CO including soluble guanylate cyclise (sGC) [27] and mitogen-activated protein kinases [19]. CORM-2 inhibits P2X4 receptors at all ATP concentrations means that this compound can be used to distinguish between P2X4 and P2X2, P2X2/3 and P2X3 receptors where CORM-2 either potentiates or has no effect. CORM-2, a well-established CO releasing molecule, is a potent, reversible and non competitive inhibitor of recombinant human P2X4 receptors. This inhibition was not mimicked by the application of a 20% CO gas solution and did not involve the generation of mitochondria-derived ROS or activation of soluble guanylate cyclase, two established mechanisms by which CO can exert cellular effects. The possible explanation of the observation is may be that the CORM-2 is independent of its ability to release CO. Also, there is no role of reactive oxygen species production in the mechanism by which CORM-2 inhibits P2X4 receptors. Although the
modus operandi remains elusive, we have demonstrated that CORM-2 is a robust, non-competitive inhibitor of P2X4 receptors.

HO-1 AND NEURODEGENERATION

In Alzheimer disease and in cases of mild cognitive impairment, there is an over-expression of HO-1 immunoreactive protein in neurons and astrocytes of hippocampus and cerebral cortex when compared among age matched cognitively integral. This protein also co-localizes with neuro-fibillary tangles, senile plaques and corpora amylacea. HO-1 is highly over-expressed in astrocytes of substantia nigra in diseases like Parkinson. Moreover, gets associated with levy bodies where dopaminergic neurons are affected. In inflammatory, cerebral infarcts, haemorrhages, multiple sclerosis play and other degenerative CNS disorders there is upregulation of HO-1 in glial cells.

In AD brain, HO-1 protein co-localizes to neurons, GFAP positive astrocytes, choroid plexus epithelial cells, ependymocytes, NFTs, senile plaques, CA and some vascular smooth muscle and endothelial cells [28,29,30]. Similarly, western blots of protein extracts derived from AD hippocampus and temporal cortex revealed strong HO-1 bands, whereas the latter were faint or undetectable in normal control preparation [30]. Importantly, significant augmentation of astroglial HO-1 expression was also documented in post-mortem brain specimens procured from individuals with mild cognitive impairment (MCI), a frequent harbinger of incipient AD [31]. In the latter, glial HO-1 immunoreactivity in temporal cortex correlated with the burden of neurofibrillary pathology. Furthermore, astroglial HO-1 expression in the temporal cortex was associated with decreased scores for global cognition, episodic memory, semantic memory, and working memory; hippocampal astroglial HO-1 expression was associated with lower scores for global cognition, semantic memory, and perceptual speed. As per the results observed, of the mild cognitive impairment (MCI), it can be concluded that the HO-1 induction play important part during initial stages in the pathogenesis of sporadic Alzheimer diseases[31]. OS provoked by the aforementioned stimuli may be responsible for the elaboration of HO-1 in the Alzheimer-diseased and MCI cerebral cortex and hippocampus. On the basis of data adduced previously (vide supra), up-regulation of HO-1 in AD/MCI-affected astroglia may perpetuate intracellular OS and contribute to pathological iron trapping, bioenergetic (mitochondrial) failure and CA formation characteristic of Alzheimer-diseased neural tissues.

Moderate HO-1 immunoreactivity is observed in dopaminergic neurons of both the normal and Parkinson-diseased substantia nigra [32]. In PD specimens, cytoplasmic Lewy bodies within affected dopaminergic neurons are prominently decorated with immunoreactive HO-1 [32,33]. The proportion of GFAP-positive astroglia that express HO-1 in the PD nigra was found to be significantly increased (77.1%) relative to age-matched control specimens (18.7%), whereas percentages of GFAP-positive astroglia co-expressing HO-1 in other subcortical nuclei, such as the caudate, putamen and globus pallidus, were relatively low in both groups [32]. DA-derived pro-oxidant intermediates, MPTP like neurotoxins generated endogenously or stemming from the environment, and microglia-derived cytokines and NO are plausible inducers of astroglial HMOX1 gene expression in the PD nigra [34].

Idiopathic PD is a late-onset movement disorder of uncertain etiology characterized pathologically by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, formation of intraneuronal fibrillar inclusions (Lewy bodies) in this cell population, and variable depletion of noradrenaline and serotonin in other brain stem nuclei. As in the case of AD, there is abundant evidence of oxidative tissue damage, bioenergy deficits and transferrin-independent iron sequestration in PD affected brain regions [32,35].
OTHER NEUROLOGICAL DISORDERS

In addition to AD and PD, HO-1 has been implicated in the path biology of numerous other degenerative and nondegenerative CNS conditions. Among the neurodegenerative disorders, the focus of this review, immunoreactive HO-1 protein localizes to diseased motor neurons in amyotrophic lateral sclerosis [36], Pick bodies in subjects with frontotemporal dementia, NFT in cases of progressive supranuclear palsy, and ballooned neurons in corticobasal degeneration [33]. On the basis of evidence discussed herein, it is conceivable that induction of HO-1 may contribute to the pathological brain iron deposition, oxidative substrate damage and mitochondrial lesions that have been documented in these late-onset human neurodegenerations [37, 38, 39, and 40]. The exquisite inducibility of the Hmox1 gene and the highly pleotropic bioactivities of all three heme degradation products have also suggested important roles for HO-1 in the pathogenesis of various neuroinflammatory [multiple sclerosis (MS), falciparum malaria], cerebrovascular (ischemic and hemorrhagic stroke), traumatic (cerebral contusions) and neuro-oncological (malignant glioma) disorders.

These conditions are not primarily neurodegenerative in nature and interested readers are referred to a previous publication addressing these topics [41]. However, a brief consideration of MS, an autoimmune demyelinating disorder of central white matter, is germane to the current discussion given recent recognition of the significant post-inflammatory, degenerative phases of the illness [42]. In a neuropathological survey [43], the fraction of GFAP-positive astrocytes expressing HO-1 (57.3%) in spinal cord plaques derived from patients with MS was noted to be substantially higher than that computed in the spinal white matter of normal subjects (15.4%). Glial HMOX1 induction in MS may be secondary to the enhanced release of IL-1b, TNF-α [43] or myelin basic protein [44] within the diseased tissues. In primary astrocyte cultures, TNF-α (20 ng/mL) or IL-1b (20 ng/mL) stimulated the accumulation of nontransferrin 55Fe by astroglial mitochondria, effects that were abrogated by co-administration of HO inhibitors, mitochondrial pore antagonists or antioxidants [43]. Thus, up-regulation of HO-1 in astrocytes may give rise to the aberrant iron deposits and electron transport chain defects reported in the vicinity of MS plaques [43,45,46] and in experimental autoimmune encephalomyelitis, a rodent model of the disease [46,47] reported increased levels of HO-1 protein in oligodendrocytes (myelin-producing cells) in early MS lesions and in astrocytes, microglia, and macrophages in acute disseminated encephalomyelitis. These investigators also provided evidence that acute HO-1 expression in cultured OLN-93 oligodendroglia confers cytoprotection in the face of H2O2 stress, whereas longer-term induction of the enzyme in these cells exacerbates oxidative injury to mitochondria and the microtubular network.
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