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Forum Review

Heme Catabolism and Heme Oxygenase in Neurodegenerative Disease

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ABSTRACT

Heme oxygenase, the rate-limiting step in heme catabolism, appears to play an important role in a number of neurodegenerative disorders, such as Alzheimer disease. Interestingly, the spatial distribution of heme oxygenase-1 expression in diseased brain is essentially identical to that of the pathological expression of tau, suggesting a key role for both in disease progression. Like heme oxygenase, the expression, phosphorylation, and aggregation of tau are regulated through signal cascades, including the extracellular signal-regulated kinases, whose activities are modulated by oxidative stress. Therefore, the expression of tau and heme oxygenase-1 in a coordinated manner likely plays a pivotal role in the cytoprotection of neuronal cells. This places heme oxygenase at the center of disease pathogenesis and offers a novel therapeutic approach targeted at either the causes or consequences of enzyme induction. *Antioxid. Redox Signal.* 6, 888–894.

INTRODUCTION

Heme oxygenase (HO), the rate-limiting step in heme degradation, is a microsomal enzyme that cleaves heme to produce biliverdin, ferric iron, and carbon monoxide (21) (Fig. 1). To date, three different HO isoforms (HO-1, HO-2, and HO-3) have been identified. HO-1 is a 32-kDa heat shock protein (47) that is inducible by numerous noxious stimuli (1). HO-2 is a constitutively synthesized 36-kDa protein that is abundant in brain and testis (63). HO-3 has a structural homology with HO-2, but its ability to catalyze heme degradation is much less than that of HO-1 or HO-2 (24). Within the brain, the majority of HO activity is attributed to the HO-2 isozyme (21, 42). The expression of HO-1 is normally very low in the brain but increases markedly after heat shock, ischemia, or glutathione depletion (11, 59, 63). As such, in the normal brain, HO-1 is restricted to select neuronal and nonneuronal cell populations in the forebrain, diencephalon, cerebellum, and brainstem (21), whereas, after heat shock or ischemia, increased HO-1 expressions were shown in neuronal and glial cells throughout the brain (21, 59, 60).

HO-1 is known as an oxidative stress-inducible protein and plays a key role in heme catabolism, in which heme, a potential prooxidant, is converted to bilirubin, an antioxidant (57). However, as HO-1 also produces other by-products, *i.e.,* carbon monoxide, a putative neuromodulator, and free iron, another potent prooxidant, it was unclear whether heme catabolism is protective or not. In this regard, increasing evidence suggests that heme degradation by HO-1 is cytoprotective against oxidative stresses. For example, several *in vivo* and *in vitro* studies have demonstrated that overexpression of HO-1 is cytoprotective against various insults (22, 39). On the other hand, HO-1 deficiency is followed by intracellular iron accumulation with increased susceptibility to oxidative stresses (3, 35, 67). Furthermore, a microsatellite polymorphism in HO-1 gene promoter (18), associated with a less inducible phenotype (14), has been reported to be responsible for various human diseases (9, 17, 43, 68).

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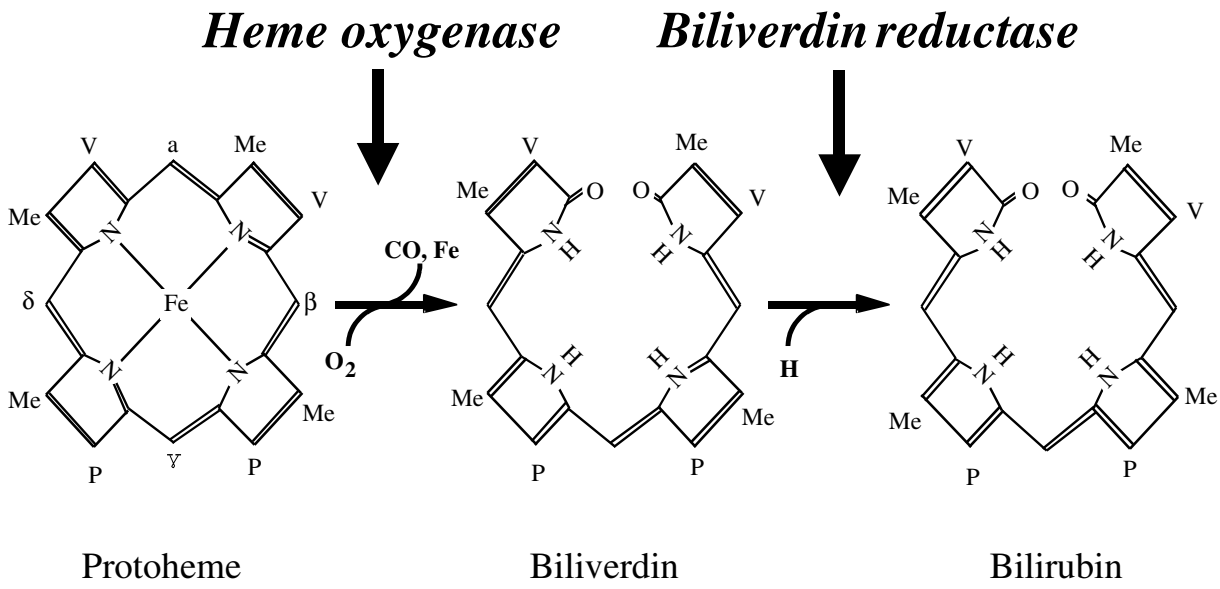

FIG. 1. Pathway of heme catabolism. Abbreviations as follows: Me, -CH_3; V, -CH=CH_2; P, -CH_2-CH_2-COOH.

INCREASED EXPRESSION OF HO-1 IN NEURODEGENERATIVE DISEASES

A host of studies clearly show that oxidative damage is associated with neurodegenerative diseases and represents one of the earliest pathological alterations in degenerating neurons (27, 28). Examples of oxidative damage include lipid peroxidation (40), nitration (51), reactive carbonyls (50), and nucleic acid oxidation (26), which are all increased in vulnerable neurons in the diseased brain. Interestingly, there is increased expression and activity of HO-1 in brains affected with neurodegenerative diseases such as Alzheimer disease (AD) (37, 45, 49), Parkinson disease (PD) (46), Pick disease, progressive supranuclear palsy, and corticobasal degeneration (7). Interestingly, HO-1 expression is coincident with pathological hallmark structures, such as neurofibrillary tangles, senile plaques, and Lewy bodies, suggesting that HO-1 plays a key role in the pathophysiological processes of neurodegeneration (21). To determine whether such increases in HO-1 lead to an increase of heme catabolism in diseased brains, we investigated the levels of bilirubin and its derivatives in cerebrospinal fluids sampled from neurodegenerative disease cases (19). Specifically, we used a monoclonal antibody 24G7, which is highly specific for bilirubins and their degraded derivatives generated by oxidative stressors (19). This enzyme-linked immunosorbent assay-based study showed a significant increase of bilirubin and its derivatives in the cerebrospinal fluid from AD cases, suggesting that the induced HO-1 is functional and that this leads to increased heme catabolism in the AD brain (19) (Fig. 2). The up-regulation of HO-1 in AD is consistent with a dysregulation of heme/iron homeostasis in the diseased brain as exemplified by alterations in iron (52), redox-active iron deposits (41), and heme and iron regulatory proteins (8, 53).

HO-1 INDUCTION IS COINCIDENT WITH CONFORMATIONAL CHANGES OF TAU

Through detailed investigations in AD brain, we found that HO-1 induction is completely coincidental with the tau conformational change recognized by the monoclonal antibody, Alz50 (Alz50-epitope) (61). The monoclonal antibody Alz50 was originally prepared against an AD brain antigen (16, 66). Characterization of the Alz50-epitope showed that it is discontinuously located in the N-terminal and C-terminal regions of tau protein and, therefore, consistent with recognition of a

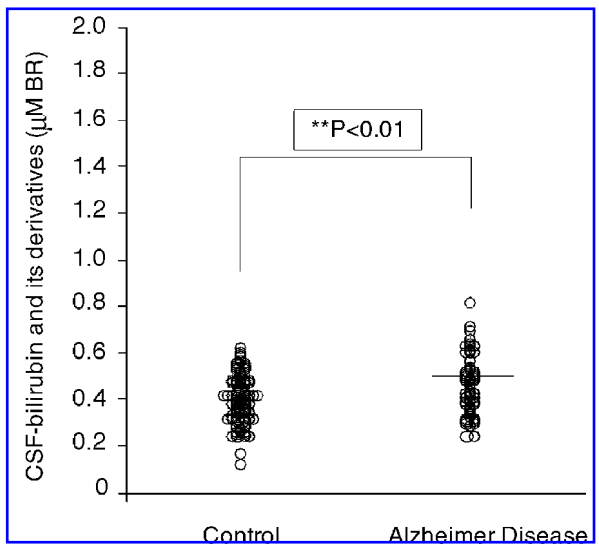

FIG. 2. Increased levels of bilirubin (BR) and its derivatives in cerebrospinal fluid of AD (modified from reference 19).

specific conformation of monomeric tau protein involving juxtaposition of N-termini and C-termini presumably stabilized by incorporation into structurally ordered tau filaments (6). Appearance of the Alz50-epitope in neurons is considered an early pathological change of tau protein preceding further pathological changes such as the formation of neurofibrillary tangles that also contain the Alz50-epitope (4). Of particular note, tau phosphorylation was additionally found in neurons lacking HO-1, indicating that phosphorylation of tau probably precedes the Alz50-epitope, and that this required an oxidative stimulus. These data provide a mechanistic link between protein phosphorylation and oxidative stress (40, 69, 73). Consistent with this interpretation, we found in *in vitro* experiments that treating normal tau protein with the reactive lipid peroxidation product, 4-hydroxy-2-nonenal (HNE), or other bifunctional aldehydes or protein modification agents capable of inducing lysine-dependent cross-linking greatly increased the presence of the Alz50-epitope. Interestingly, bilirubin can bind to proteins through lysine residues, the primary site of oxidative adduction. Therefore, the increased bilirubin may prevent cross-linking of tau by capping lysine residues and thus play a key neuroprotective role in AD. It is intriguing that other neurological conditions where tau accumulation and the Alz50-epitope are found also show increases in HNE adducts, *e.g.,* progressive supranuclear palsy and fronto-temporal dementia (unpublished observations). As tau phosphorylation is accelerated by HNE (23), our data suggest that oxidative stress and attendant modification of tau by products of oxidative stress, including HNE as well as other cytotoxic carbonyls, may trigger cytoskeletal pathology in AD (33). Although our data show that HO-1 plays a pivotal role in tau modification by lipid peroxidation products, it was also demonstrated that only 20–40% of HNE-positive neurons coexpress HO-1, suggesting that the induction of HO-1 is not simply regulated by oxidative stress. Among a host of known stimulants, heme is one of the most effective inducers of HO-1 (31). In AD, mitochondria, rich in heme molecules, show profound abnormalities (15), and it is therefore plausible that HO-1 is induced by excess heme release from damaged mitochondria. Furthermore, it is intriguing to consider that HO-1 induction may be a response to cytochrome *c* released from mitochondria that protects neurons from apoptosis (48).

POSSIBLE INTERACTION BETWEEN HO-1 AND TAU PROTEIN

To explore possible interactions between HO-1 and tau proteins, we transfected sense and antisense human HO-1 cDNA into human neuroblastoma cells (62). The transfection of the DNA constructs did not affect the morphology or growth rate of the cells. Cells transfected with the sense HO-1 cDNA exhibited a severalfold induction of HO-1 transcripts and protein, which was mirrored in a more than threefold increase in HO activity (62). Overexpression of the HO-1 gene considerably enhanced resistance of the cells to oxidative injury produced by hydrogen peroxide, which is consistent with other reports showing that HO-1 overexpression is cytoprotective in neuronal and nonneuronal cells (2). On the other hand, in the antisense transfected cells, there was residual HO activity below the basal level that is probably due to the expression of intrinsic HO-2. In fact, HO-2 expression was not affected by transfection of the HO-1 cDNA constructs. One notable effect of HO-1 overexpression was that expression of tau protein was specifically suppressed. This suppression was partially alleviated by treatment with an HO inhibitor, suggesting that the regulation of tau expression might depend on HO activity. The detailed regulatory mechanism for tau gene expression is still unknown, but recently, extracellular signal-regulated kinases (ERKs), which are prominent members of the mitogen-activated protein kinase (MAPK) family and are elevated in AD (34, 62, 70, 71), were shown to induce tau promoter activity (62). In our study, HO-1 overexpression decreased the phosphorylated (activated) forms of ERK-1 and ERK-2, which is consistent with the observed suppression of tau expression. When the HO inhibitor was added, the phosphorylated form of ERK-1 increased over the control levels, suggesting that ERK-1 activation was down-regulated by the increased HO activity and that the HO inhibitor suppressed not only the overexpressed HO-1 activity, but also the intrinsic HO-2 activity. Although the level of the activated form of ERK-1 was increased and that of ERK-2 was restored to control levels by the addition of HO inhibitor, only partial recovery of tau protein expression was observed. This suggests that tau gene expression is not directly controlled by ERK cascades. In fact, tau has been classified as a "late gene," and the effect of MAPK cascades on its expression is thought to be indirect and mediated by additional downstream transcription factors (62). Interestingly, changes in cell growth and morphology were not observed in HO-1-overexpressing cells even though tau protein expression was suppressed. This implies that other molecules can substitute for the tau protein, which has also been suggested in previous reports using a cell culture model and in knockout mice (13). Overall, these findings again provide a compelling link between phosphorylation, here of tau, and oxidative stress and, furthermore, suggest a synergistic relationship between tau phosphorylation and oxidative balance (55, 69, 73).

HEME CATABOLISM AND IRON METABOLISM IN NEURODEGENERATION

The HO-1 knockout mice (35, 36) and the reported human case (67) with mutant HO-1 gene display phenotypes with severe anemia and iron deposition in certain tissues, as well as general vulnerability to oxidaitve stresses, suggesting that heme catabolism plays a key role in iron metabolism, including efflux of non-heme iron from cytoplasm (44). As free iron can catalyze Haber–Weiss and Fenton reactions causing the formation of highly toxic radicals, such as peroxynitrite, iron accumulation will increase a bulk of oxidative stress in tissues. Because the deposition of non-heme iron within affected neuronal tissues is a common feature among AD, PD, and other neurodegenerative diseases (54), possible roles of heme catabolism in iron metabolism have been attracting at-

tention of researchers. Using *in vitro* models, Shipper (44) showed that HO-1 is responsible not only for extracellular export of iron, but also for intramitochondrial sequestration of iron. We demonstrated that overexpression of HO-1 did not affect the levels of ferritin expression, suggesting that there were no changes of free iron levels in cytoplasm following the HO-1 overexpression. Accumulating data indicate that HO accelerates iron efflux from the intracellular compartment, possibly through the ATP-dependent iron transport system (3) and/or the formation of CO–Fe complex (65).

PHYSIOLOGICAL ROLES OF CARBON MONOXIDE

Another product of heme catabolism, carbon monoxide, is a potent neurotransmitter and plays a role in cellular signal transduction (5). Through guanyl cyclase–cyclic GMP and MAPK pathways, it was demonstrated to promote vasodilation (64), to inhibit inflammatory process (32), and to cause G1/S growth arrest of the cell cycle (10). Moreover, recent data demonstrate that low concentration of carbon monoxide exposure by itself is antiapoptotic and cytoprotective against oxidative stress (12, 20, 56), although it is a well known highly toxic gas. In AD brain, microglial activation was indicated to facilitate disease processes, and certain antiinflammatory agents were shown to have therapeutic potential. Moreover, aberrant cell-cycle activation was also described in brains affected with neurodegenerative diseases (25, 29, 30, 38, 54, 72). Thus, heme catabolism, which is the only metabolic pathway to produce carbon monoxide *in vivo*, may attenuate these pathological processes by inhibiting the inflammatory process and arresting aberrant activation of neuronal cells.

CONCLUSIONS

Taken together, the products of heme catabolism seem to be neuroprotective, and its decrease may lead to increased vulnerability against oxidative stresses. We found that the poly(GT) sequence present in the HO-1 gene promoter is highly polymorphic and analyzed their allele frequencies in AD and PD (18). However, we failed to find any associations between a certain allele for this polymorphism and neurodegenerative diseases, such as AD and PD (18). More recently, through yeast two-hybrid screening, amyloid β protein precursor (AβPP) was shown to bind HOs and to inhibit their activities (58). Furthermore, it was demonstrated that the mutations of AβPP linked to familial AD provided greater inhibition of HO activity compared with wild-type AβPP (58). These data suggest that although changes in the HO-1 gene may not be directly linked to the pathogenesis of these diseases, heme catabolism likely plays pivotal roles in neuronal survival and cytoprotection against aging and environmental insults. Further study of neurodegenerative diseases by exploring this ubiquitous but unique metabolic pathway will shed light on novel fields and methods for modulation of disease processes.

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ABBREVIATIONS

AD, Alzheimer disease; AβPP, amyloid β protein precursor; ERK, extracellular signal-regulated kinase; HNE, 4-hydroxy-2-nonenal; HO, heme oxygenase; MAPK, mitogen-activated protein kinase; PD, Parkinson disease.

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