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Heme oxygenase-1: role in brain aging and neurodegeneration☆

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Abstract

The mechanisms responsible for excessive iron deposition and mitochondrial insufficiency in the aging and degenerating nervous system remain poorly understood. Heme oxygenase-1 (*HO-1*) is a 32 kDa stress protein that degrades heme to biliverdin, free iron and carbon monoxide. Our laboratory has shown that cysteamine, dopamine, β-amyloid, IL-1β and TNF-α up-regulate *HO-1* followed by mitochondrial sequestration of non-transferrin-derived ^{55}Fe in cultured rat astroglia. In these cells and in rat astroglia transfected with the human *HO-1* gene, mitochondrial iron trapping is abrogated by the *HO-1* inhibitors, tin-mesoporphyrin and dexamethasone. We determined that *HO-1* immunoreactivity is enhanced greatly in neurons and astrocytes of the hippocampus and cerebral cortex of Alzheimer subjects and co-localizes to senile plaques and neurofibrillary tangles (NFT). *HO-1* staining is also augmented in astrocytes and decorates neuronal Lewy bodies in the Parkinson nigra. Collectively, our findings suggest that *HO-1* over-expression contributes to the pathological iron deposition and mitochondrial damage documented in these aging-related neurodegenerative disorders. We recently observed that, paradoxically, *HO-1* mRNA levels are markedly suppressed in peripheral lymphocytes of patients with early sporadic Alzheimer disease and may thus provide a useful biological marker of this condition.

Keywords: Aging; Alzheimer disease; Amyloid; Astrocyte; Dopamine; Heme Oxygenase-1; Iron; Mitochondria; Oxidative stress; Parkinson disease

1. Introduction

Neuronal degeneration, gliosis, mitochondrial insufficiency and the deposition of

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non-heme iron within affected neural tissues are characteristic features of Parkinson's disease (PD) and Alzheimer disease (AD) and also occur, to a lesser extent, in the course of normal brain aging. The excessive sequestration of redox-active iron in the substantia nigra of patients with idiopathic PD and in the basal forebrain and association cortices of AD victims has been implicated as a major generator of damaging reactive oxygen species (ROS) in these tissues. Yet, the mechanisms responsible for this pathological iron deposition remain poorly understood. In both the PD and AD brain, regional concentrations of transferrin binding sites remain unchanged or vary inversely with the elevated iron stores. These observations suggest that the transferrin pathway of iron mobilization, critical for normal iron delivery to most peripheral tissues, contributes little to the pathological deposition of brain iron in these aging-related neurodegenerative conditions (Schipper, 1999). The potential role of alternative iron transport mechanisms, such as that mediated by lactoferrin and the lactoferrin receptor, is reviewed elsewhere (Schipper, 1998). In this paper, evidence implicating the enzyme, heme oxygenase-1 (*HO-1*) in senescence-related glial iron sequestration and in the pathophysiology of PD and AD is considered.

2. Heme oxygenase-1

HO-1 is a 32 kDa member of the stress protein superfamily that catalyzes the oxidative degradation of heme to biliverdin in brain and other tissues (Tenhunen et al., 1969; Ewing and Maines, 1991). The *HO-1* gene has a heat shock consensus sequence and AP1, AP2 and NFκB binding sites in its promoter region and is rapidly upregulated by oxidative stress, metal ions, amino acid analogues, sulfhydryl agents, pro-inflammatory cytokines and hyperthermia (Applegate et al., 1991). In response to oxidative challenge, induction of *HO-1* may protect cells by promoting the catabolism of pro-oxidant metalloporphyrins, such as heme, to bile pigments (biliverdin, bilirubin) with free radical scavenging capabilities (Stocker et al., 1987; Doré et al., 1999). On the other hand, free iron and carbon monoxide (CO) liberated in the course of heme catabolism may, under certain circumstances, exacerbate intracellular oxidative stress and mediate injury to mitochondrial membranes (Zhang and Piantadosi, 1992). This disparate behaviour has fostered lively controversy as to whether *HO-1* up-regulation in the diseased nervous system subserves a cytoprotective function (Dwyer et al., 1995; Panahian et al., 1995; Takeda et al., 2000) or is an agent for further neuroendangerment (Panizzon et al., 1996; Koeppen and Dickson, 1999; Schipper, 1999). These opposing views may not be mutually exclusive; in a given neuropathological condition, the extent and duration of *HO-1* induction and the status of the local redox microenvironment may determine whether the pro-oxidant potential of liberated iron/CO or the antioxidant benefits of a diminished heme:bilirubin ratio prevail (Galbraith, 1999).

3. *HO-1* and glial iron sequestration

Our laboratory has been investigating mechanisms responsible for the biogenesis of iron-rich cytoplasmic inclusions in astrocytes of the senescent and degenerating mammalian CNS. We determined that oxidative stress is a 'final common pathway' mediating the transformation of normal glial mitochondria to iron-laden cytoplasmic granules and

corpora amylacea (cellular inclusions observed in aging neural tissues) in hippocampus and other subcortical brain regions in situ and in primary astroglial cultures exposed to the sulfhydryl agent, cysteamine(CSH; reviewed in Mydlarski and Schipper, 1998; Mydlarski et al., 1998; Schipper, 1998). Using the latter in vitro model, we showed that the pro-oxidant effects of CSH (Mydlarski et al., 1993; Wang et al., 1995), H_2O_2, menadione, dopamine (Schipper et al., 1999), tumour necrosis factor α (TNFα), interleukin-1β (IL-1β; Mehindate et al., 1998) and β-amyloid$_{40/42}$ (Ham and Schipper, 2000) rapidly promote up-regulation of astroglial *HO-1* mRNA and protein levels followed by sequestration of ^{59}Fe or ^{55}Fe by the mitochondrial compartment. These effects were only demonstrable when $^{59}FeCl_3$, but not ^{59}Fe–diferric transferrin, served as the metal donor. The latter observation is consistent with the aforementioned conclusion that transferrin and its receptor probably play little or no role in pathological iron trapping characteristic of aging and degenerating neural tissues. We subsequently demonstrated that sequestration of mitochondrial ^{55}Fe in astroglial cultures exposed to dopamine (Schipper et al., 1999) or β-amyloid$_{40/42}$ (Ham and Schipper, 2000) is attenuated by co-administration of tin-mesoporphyrin (SnMP), a competitive inhibitor of heme oxygenase activity, or dexamethasone, a transcriptional suppressor of the *HO-1* gene. Moreover, we showed that over-expression of *HO-1* in cultured rat astroglia by transient transfection of full-length human *HO-1* cDNA promotes trapping of non-transferrrin-bound ^{55}Fe by the mitochondrial compartment. As in the case of the oxidatively-stressed astroglia, administration of SnMP or dexamethasone abrogated mitochondrial iron sequestration in the *HO-1* transfected cells (Schipper et al., 1999). Taken together, our findings argue that up-regulation of *HO-1* in oxidatively-challenged glial cultures and in astrocytes of the aging subcortical brain is necessary and sufficient for progressive deposition of non-transferrin-derived iron within the mitochondrial compartment. In astroglia exposed to dopamine, mitochondrial iron uptake can be also be blocked by co-administration of cyclosporin A or trifluoperazine, inhibitors of the mitochondrial permeability transition pore (Schipper et al., 1999). Thus, in 'stressed' astroglia, opening of the permeability transition pore appears to mediate the influx of low-molecular iron into the mitochondrial matrix. Finally, over-expression of *HO-1* in cultured glia by CSH/dopamine stimulation or transient transfection with human *HO-1* cDNA results in late, compensatory induction of the manganese superoxide dismutase gene, an effect that can be attenuated by co-treatment with ascorbate and other antioxidants (Manganaro et al., 1995; Frankel et al., 2000). Thus, in astroglia, up-regulation of *HO-1* may perpetuate intracellular ROS generation, oxidative mitochondrial injury and iron deposition within this organelle long after the effects of exogenous stressors have dissipated. Using electron spin resonance spectroscopy, we demonstrated that the glial mitochondrial iron behaves as a non-enzymatic (or pseudo-) peroxidase activity that is capable of oxidizing dopamine and other catechol-containing compunds to potentially neurotoxic semiquinone radicals (Schipper et al., 1991; Schipper, 2000). That free radical generation within this glial compartment may be harmful to the nearby neuronal constituents is underscored by our observation that PC12 cells grown atop iron-rich astrocytes (a senescent glial phenotype) are far more vulnerable to dopamine/H_2O_2 — related killing than PC12 cells co-cultured with young, healthy (iron-poor) astroglia (Frankel and Schipper, 1999). Patterns of *HO-1* expression in PD and AD and their implications for the pathogenesis of these disorders are discussed in the following sections.

4. *HO-1* and Parkinson disease

Immunohistochemical techniques were employed to determine whether *HO-1* is over-expressed, and the cellular distribution of this enzyme, in post-mortem brain specimens derived from PD subjects relative to specimens procured from subjects with no history of neurologic illness and no specific neuropathological evidence consistent with parkinsonism (Schipper et al., 1998): (i) Neurons: neurons exhibiting *HO-1* staining were rarely encountered in the hippocampus, caudate, putamen and globus pallidus of both the control and PD brains. When present, *HO-1* immunoreactivity appeared as a faint, diffuse precipitate largely confined to the perikarya and apical dendrites with sparing of the cell nuclei. In the substantia nigra (pars compacta) of both PD and control specimens, moderate *HO-1* immunostaining was consistently observed in neuromelanin-containing (dopaminergic) neurons. No apparent differences in the intensity of cytoplasmic *HO-1* staining in these neurons could be discerned between PD and control cases. However, Lewy bodies, characteristic cytoplasmic inclusions present only in the PD specimens, exhibited strong *HO-1* staining in their peripheries. Furthermore, *HO-1* immunoreactivity in the nigral neuropil of PD specimens was generally more prominent than that observed in the controls. As in the case of the other brain regions surveyed, *HO-1* immunoreactivity in non-dopaminergic neurons of the substantia nigra was faint or non-existent. (ii) Astrocytes: using dual immunolabeling for *HO-1* and the astrocyte-specific marker, glial fibrillary acidic protein (GFAP), small numbers of *HO-1*-positive astrocytes were seen randomly interspersed among a much larger pool of GFAP-positive/*HO-1*-negative cells in the hippocampus, basal ganglia and substantia nigra of the non-PD specimens. In contrast, a majority of GFAP-positive astrocytes in the PD-affected nigra, but not in other brain regions, exhibited robust *HO-1* immunoreactivity. The fraction of GFAP-positive astrocytes expressing detectable *HO-1* in the PD nigra (~77%) was significantly greater than that computed in the nigra of control specimens (~19%). (iii) Other cell types: in control, PD and AD specimens, intense *HO-1* immunoreactivity was observed in choroid plexus epithelial cells, the apical cytoplasm of ependymocytes and in many vascular endothelial cells. In these tissues, no differences in *HO-1* staining intensity were noted between the clinical groups.

Because *HO-1* expression is a sensitive marker of cellular oxidative stress, our data suggest that dopaminergic neurons in the human substantia nigra are exposed to increased levels of oxidative stress in the course of normal aging relative to other (*HO-1*-negative) brain cell populations. This interpretation is consistent with reports that aging-dependent depletion of nigral dopaminergic neurons may be occurring at an accelerated pace relative to other neuronal populations (Mann, 1994). Furthermore, neuromelanin formation in nigral dopaminergic neurons is largely derived from polymerization of oxidized catecholamine moieties attesting to the robust activity of oxidative metabolic activity in these cells (Fornstedt et al., 1989). The finding that, in PD specimens, *HO-1* is a constituent of neuronal Lewy bodies supports the contention that oxidative processes play an important role in their biogenesis, as others have previously conjectured (Montine et al., 1995). Perhaps most importantly, the massive up-regulation of *HO-1* in astrocytes of the PD nigra, in conjunction with the in vitro data discussed in Section 3, suggest that heme-derived elemental iron and CO contribute substantially to the abnormal pattern of iron

deposition and oxidative mitochondrial lesions reported in the brains of PD subjects (Beal, 1995; Reichmann and Riederer, 1994).

5. *HO-1* and Alzheimer pathology

Using immunolabeling techniques, we observed profound over-expression of *HO-1* protein in brain specimens derived from subjects with neuropathologically-proven AD relative to controls with no history of neurological illness matched for age and post-mortem interval (Schipper et al, 1995). Neurons: as described in Section 4, the vast majority of neurons in the control material exhibited very weak or no *HO-1* immunoreactivity. Similarly, *HO-1* staining appeared as a very faint 32 kDa band or was absent in Western blots of protein extracts derived from control hippocampus, temporal cortex and subcortical white matter. These data indicate that constitutive expression of this stress protein is very low in the course of normal brain aging, findings consistent with earlier observations in adult rats (Ewing et al., 1992). In the AD brains, numerous neurons throughout the hippocampus (pyramidal and dentate gyrus granule cells) and temporal cortex (layers II–V) exhibited intense, cytoplasmic *HO-1* immunoreactivity. Although many neurons without overt neurofibrillary pathology were also strongly immunopositive, *HO-1* expression was most conspicuous in neurons containing NFT.

In sections dually labeled for *HO-1* and tau-2, we demonstrated consistent and robust co-localization of *HO-1* to the abundant NFT observed in the AD specimens. Occasional NFT encountered in the control material were also intensely *HO-1*-positive. As in the case of the NFT, senile plaques (SP) were strongly immunoreactive for *HO-1* in AD (and control) tissues stained for both *HO-1* and β-amyloid. In sections co-labeled with anti-*HO-1* and anti-synaptophysin antisera, *HO-1*-positive neurites were seen in and around the periphery of SP. Because *HO-1* is a very sensitive marker of oxidative stress, the latter observation supports the notion that β-amyloid deposits within SP may subject nearby neuropil elements to oxidative stress. Astrocytes: As in the PD study described above, some AD and control brain sections were dual labeled for *HO-1* and the astrocyte marker, GFAP. *HO-1*-positive astrocytes were infrequently seen in the hippocampus and temporal cortex derived from the control specimens. For example, only 6.8% of GFAP-positive astrocytes co-expressed immunodetectable *HO-1* in control hippocampus. In striking contrast, 86% of GFAP-positive astrocytes in the AD hippocampus co-expressed *HO-1*. In both control and AD cases, no consistent topographical relationship between *HO-1*-positive glia and SP was noted. In the AD substantia nigra, a region largely spared by the disease, glial *HO-1* immunoreactivity was minimal and not significantly different from control values.

Up-regulation of *HO-1* in AD brain is consistent with the induction of other stress proteins, such as heat shock protein (hsp)27, hsp72, αB-crystallin and ubiquitin, reported in this condition (Perez et al., 1991; Wang et al., 1991; Lowe et al., 1992; Renkawek et al., 1993). Oxidative stress has been amply documented in AD-affected brain tissues (Mattson, 1997) and may be responsible for induction of *HO-1* and other stress proteins in these patients (Schipper et al., 1995). As discussed above, upregulation of *HO-1* may confer cytoprotection to vulnerable neurons by catalyzing the degradation of pro-oxidant heme

moities to bile pigments (biliverdin, bilirubin) with antioxidant capabilities (Stocker et al., 1987; Doré et al., 1999). In a recent paper, Smith and colleagues (Takeda et al., 2000) showed that tau expression is down-regulated in a neuroblastoma cell line transfected with human *HO-1* cDNA. On the basis of this observation, the authors proposed that similar *HO-1*/tau interactions in situ may be beneficial in AD by limiting the substrate (tau) for pathological hyperphosphorylation (a precursor of NFT formation) in this disease. However, this conclusion remains highly conjectural because tau phosphorylation was not addressed in this study and tau gene suppression has not, to our knowledge, been demonstrated in AD brain tissues. On the contrary, down-regulation of tau may compromise the integrity of the cytoskeleton and thereby predispose to cytopathological changes.

A significant body of evidence suggests that sustained up-regulation of *HO-1* in AD brain may facilitate the development of some of the disease's neuropathological manifestations: (i) As discussed above, *HO-1* over-expression in cultured astroglia augments sequestration of non-transferrin-derived iron by the mitochondrial compartment (Schipper, 1999; Schipper et al., 1999). Amyloid peptides stimulate *HO-1* expression and mitochondrial iron trapping in astrocytes, and the latter effect is attenuated by inhibitors of *HO-1* (Ham and Schipper, 2000). Thus, chronic over-expression of *HO-1* in the AD brain, possibly in response to excessive amyloid provocation, may account for the (transferrin receptor-independent) iron overload and mitochondrial insufficiency observed in this disorder (Reichmann and Riederer, 1994; Beal, 1995). Using a co-culture paradigm, we demonstrated that sequestration of mitochondrial iron by the glial substratum greatly enhances the vulnerability of neuron-like PC12 cells to oxidative injury (Frankel and Schipper, 1999). To the extent that similar glial-neuronal interactions operate in situ, it is conceivable that pathological iron accumulation by the glial compartment may endanger nearby neuronal constituents in the brains of AD subjects. (ii) Transient transfection of human *HO-1* cDNA into rat astroglia stimulates late, compensatory induction of the manganese superoxide dismutase gene. The latter can be significantly attenuated by antioxidant treatment indicating that *HO-1* over-expression, at least in astroglia, promotes the generation of intracellular oxidative stress (Frankel et al., 2000). Free iron and CO, products of HO-mediated heme degradation, are likely culprits mediating oxidative stress in these cells (Zhang and Piantadosi, 1992) and, analogously, in neurons and glia over-expressing *HO-1* in AD-afflicted brain tissues. (iii) By augmenting cyclic guanosine monophosphate in olfactory neurons (Verma et al., 1993), regulating the secretion of corticotropin-releasing hormone (Parks et al., 1994), perturbing central vasoregulatory mechanisms (Marks et al., 1991) and modulating hippocampal long-term potentiation (Stevens and Wang, 1993; Zhuo et al., 1993), CO may also facilitate the development of cognitive (Liebson and Albert, 1994), olfactory (Doty, 1991) and neuroendocrine (Sapolsky, 1992) disturbances characteristic of AD.

6. *HO-1*: A biological marker of sporadic AD

After observing excessive levels of immunoreactive *HO-1* protein in the brains of AD subjects (vide supra), we wondered whether *HO-1* expression would be similarly increased in peripheral tissues derived from patients with early sporadic AD relative to

age-matched, normal elderly controls (NEC), patients with various dementing and non-dementing neurological conditions (e.g. PD, stroke, Huntington's disease) and several chronic medical disorders (e.g. rheumatoid arthritis, chronic active hepatitis; Schipper et al., 2000). Contrary to hypothesis, we discovered that *HO-1* protein levels, determined by ELISA, were significantly lower in the plasma of patients with probable early sporadic AD and in the CSF of neuropathologically-definite AD relative to NEC values. Moreover, lymphocyte *HO-1* mRNA levels, measured by Northern blotting and laser densitometry, were highly suppressed in *early* sporadic AD in comparison with NEC and the neurological/medical control groups. These data indicate that the decreased *HO-1* protein levels in AD plasma/CSF reflect diminished systemic production of the enzyme as opposed to accelerated degradation of *HO-1* in the circulation. The mechanism responsible for the down-regulation of *HO-1* in AD lymphocytes remains unclear. Kimpara et al. (1997) did not identify over-representation of specific *HO-1* gene polymorphisms in patients with AD, arguing that primary genetic determinants of *HO-1* are probably not responsible for reduced levels of this enzyme in AD peripheral tissues.

Measurement of *HO-1* mRNA levels in peripheral lymphocytes may provide a useful biological marker of sporadic AD as defined in a recent consensus report on this topic (Trojanowski and Growdon, 1998): (1) Diminished *HO-1* mRNA levels exhibit high sensitivity (88%) and moderate to high specificity (75%) in differentiating sporadic AD from NEC and other dementing conditions (Schipper et al., 2000). (2) Lymphocyte *HO-1* mRNA levels are suppressed in *early* sporadic AD and in some individuals with Minimal Cognitive Impairment (MCI), a high risk factor for the development of incipient AD (ibid). (3) Blood *HO-1* abnormalities may reflect some fundamental process of the disease because: (a) *HO-1* is over-expressed and co-localizes to SP and NFT in AD brain (Smith et al., 1994; Schipper et al., 1995); and (b) central up-regulation of *HO-1* may foster aberrant iron deposition and mitochondrial lesions characteristic of AD-affected brain tissues (Schipper et al., 1999). (4) Lymphocyte *HO-1* mRNA assays are relatively non-invasive and inexpensive and could be made available in many hospital laboratories.

7. Conclusions

Induction of the *HO-1* gene in AD- and PD-affected brain regions constitute further evidence that these tissues are subjected to chronic oxidative stress over and above that experienced in the course of normal brain aging. The downstream effects and physiological significance of this *HO-1* response, however, remain open to interpretation. One view maintains that *HO-1* is 'good for the brain' insofar as the intracellular catabolism of pro-oxidant heme molecules to antioxidant bile pigments may help restore a more favourable redox microenvironment in these neurodegenerative conditions. On the other hand, data reviewed in this paper suggest that chronic over-expression of *HO-1*, and attendant liberation of intracellular free iron and CO, may contribute to the aberrant patterns of brain iron deposition and mitochondrial insufficiency documented in AD, PD and other aging-related neurodegenerative disorders. In contradistinction to its robust expression in the affected brain, *HO-1* mRNA and protein levels are suppressed in the blood of patients with early sporadic AD and some individuals with MCI. These latter observations suggest

that *HO-1* dysregulation may reflect some fundamental aspect of the pathophysiology of early sporadic AD, and implicate *HO-1* as a potentially useful biological marker of this condition.

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