PERSPECTIVE

Is glial heme oxygenase-1 suppression in neurodegenerative disorders permissive for neural repair?

'Core' neuropathology of degenerative central nervous system (CNS) disorders

The common human neurodegenerative disorders (Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis, *etc.*) vary with respect to risk factors, ages of onset, sex predilections, neuraxial regions affected, hallmark cellular inclusions, behavioral and neurological symptoms, and responses to treatment. Despite these differences, there appears to be a set of 'core' neuropathological features shared among these and related entities. Common to these conditions are 1) pathological deposition of non-transferrin bound iron, 2) oxidative stress and associated protein, lipid and nucleic acid modifications, 3) mitochondrial membrane damage and bioenergetic failure, and 4) macroautophagy in the affected neural tissues. To the extent that endogenous neural repair is achievable in the context of chronic neurodegeneration, abrogation of this common neuropathological tetrad may translate into functional recovery (and not merely arrested decline) in patients with degenerative brain disorders. Based on the results of human neuropathological surveys, animal models and tissue culture experiments, we posited that these core features may constitute a single neuropathological 'lesion', with self-reinforcing servomechanisms (positive feedback loops) driving the degenerative process and ensuring that the advent of one component obligates the appearance of the others (Schipper et al., 2009). Moreover, we adduced evidence that up-regulation of the stress protein, heme oxygenase-1 (HO-1) in astrocytes is a pivotal transducer of various noxious stimuli into precisely this core neuropathological signature.

HO-1 and neurodegeneration: (1) *HO-1 molecular biology*- Heme oxygenases are endoplasmic reticulum enzymes that oxidize heme to biliverdin, free ferrous iron and carbon monoxide (CO; **Figure 1**). Biliverdin reductase further catabolizes biliverdin to the bile pigment, bilirubin. Mammalian cells express inducible HO-1 (a.k.a. HSP32) and constitutive HO-2 which differ with regard to molecular weight, electrophoretic mobility, tissue distribution, regulation, and antigenicity. The half-lives of HO-1 mRNA and protein are approximately 3 hours and 15–21 hours, respectively (Dennery, 2000). In humans, the *ho-1* gene (*Hmox1*) is located on chromosome 22q12 and consists of four introns and five exons. The regulatory region of the mammalian *HMOX1* gene features a 500-bp promoter, a proximal enhancer, and two or more distal enhancers. The *Hmox1* promoter exhibits AP-1, AP-2, nuclear factor kappa B (NF-κB), and HIF-1 binding sites, as well as heat shock consensus (HSE) sequences, metal response elements (MtRE, CdRE), and stress response elements (StRE). This large array of consensus sequences renders the gene exquisitely sensitive to induction by diverse pro-oxidant and inflammatory stimuli including heme, dopamine (DA), tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), cysteamine (CSH), β-amyloid, H_2O_2, hyperoxia, UV light, heavy metals and lipopolysaccharide. Nrf2 transcription factor binding to Maf response elements (MARE) in the *Hmox1* promoter and repression of the gene by the heme-regulated protein, Bach1 are key control mechanisms for HO-1 induction and homeostasis in stressed brain and other organs. In addition, a 56-bp sequence (STAT-3 acute-phase response factor binding site) within the *Hmox1* promoter confers susceptibility to transcriptional suppression by glucocorticoids (Lavrovsky et al., 1996).

All mammalian cells metabolize heme. Oxidative stress (OS) may transiently augment the intracellular "free heme pool" by stimulating the breakdown of hemoproteins such as cytochromes, myoglobin, peroxidases and respiratory burst enzymes. In many stressed cells, the up-regulation of HO-1 confers protection by accelerating the degradation of pro-oxidant heme to the radical-scavenging bile pigments, biliverdin and bilirubin. Co-stimulation of apoferritin synthesis, a major iron sequestration pathway, may prevent toxicity resulting from the intracellular liberation of heme-derived ferrous iron (Dennery, 2000; Ryter and Tyrrell, 2000). Alternatively, in cultured astrocytes and other cell types, heme-derived iron and CO may exacerbate intracellular OS and substrate damage by promoting the generation of reactive oxygen species within the mitochondrial compartment (Schipper et al., 2009). Indeed, there is ample literature implicating both neuroprotective and neuroendangering roles for HO-1 in intact animals and in tissue culture (reviewed in (Schipper et al., 2009)). Examples of HO-1-mediated neuroprotection include the following: (i) Cerebellar granule cells derived from HO-1 overexpressing transgenic mice and neuroblastoma cells transfected with *Hmox1*cDNA are relatively resistant to glutamate- and H_2O_2-mediated oxidative damage; and (ii) HO-1 transgenic mice subjected to cerebral ischemia, brain or spinal cord trauma, or excitotoxin exposure exhibit smaller infarct sizes and lower biochemical indices of neural injury. Evidence supporting neurodystrophic actions of HO-1 include: (i) diminished tissue necrosis and edema by metalloporphyrin suppression of heme oxygenase activity following focal cerebral ischemia or experimental intracerebral hemorrhage in rodent models; and (ii) the detrimental impact of chronic glial *Hmox1* induction in senescent and degenerating neural tissues as discussed in the following sections. The intensity and temporal pattern of HO-1 expression, the chemistry of the local redox microenvironment, species differences, and various experimental parameters may determine whether free radical damage accruing from the intracellular release of iron/CO or the antioxidant benefits of a suppressed heme: bilirubin ratio predominate.

(2) *HO-1 in human brain aging and degeneration*- In the normal adult human brain, HO-1 immunoreactivity is detectable in small numbers of scattered neurons, astrocytes and microglia; in the olfactory neuroepithelium; in choroid plexus epithelial cells and ependymocytes; and in cerebrovascular smooth muscle and endothelial cells. Neuroglia immunoreactive for HO-1 in the normal human brain accumulate progressively with advancing aging. In the AD-affected cerebral cortex and hippocampus, the proportion of GFAP-positive astrocytes expressing HO-1 increases dramatically relative to age-matched, non-demented controls. In one study, 86% of glial fibrillary acidic protein (GFAP)-positive astrocytes residing within the AD hippocampus exhibited HO-1 immunoreactivity, whereas the fraction of hippocampal astroglia expressing HO-1 in age-matched normal tissue was in the range of only 6–7%. 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 preparations. There is also substantial augmentation of astroglial HO-1 expression in the brains of individuals with mild cognitive impairment (MCI), a frequent harbinger of incipient AD (Schipper et al., 2009). Glial HO-1 immunoreactivity in the MCI temporal cortex correlated with the burden of neurofibrillary pathology and was associated with decreased scores for global cognition, episodic memory, semantic memory and working memory. Similarly, hippocampal astroglial HO-1 expression was associated with lower scores for global cognition, semantic memory and perceptual speed. The MCI findings indicate that glial *HMOX1* induction is a relatively early event in the pathogenesis of sporadic AD. In PD,

HO-1 decorates the pathognomonic Lewy bodies within affected dopaminergic perikarya. Moreover, the fraction of GFAP-positive astroglia that expressed HO-1 in the PD substantia nigra (77.1%) was significantly greater than that of age-matched control specimens (18.7%), whereas the proportion of astrocytes co-expressing HO-1 in other subcortical nuclei, such as the caudate, putamen and globus pallidus, was low in both the PD and control specimens. MPTP-like environmental neurotoxins, microglia-derived cytokines and NO, and redox-active metabolites of dopamine, may be responsible for astroglial *HMOX1* gene induction in the PD nigra (Schipper et al., 2009).

HO-1 may also exert important biological effects in numerous other degenerative and non-degenerative CNS disorders. Among the neurodegenerative conditions, HO-1 immunoreactivity localizes to diseased motor neurons in *amyotrophic lateral sclerosis*, ballooned neurons in *corticobasal degeneration*, neurofibrillary tangles in *progressive supranuclear palsy*, and Pick bodies in *frontotemporal dementia*. The remarkable sensitivity of the *HMOX1* gene to induction by noxious stimuli, and the potent bioactive properties of the heme degradation products, have also implicated HO-1 in the pathogenesis of various traumatic (*cerebral contusions*), cerebrovascular (*ischemic and hemorrhagic stroke*), neuro-oncological (*malignant glioma*) and neuroinflammatory (*multiple sclerosis, falciparum malaria*) afflictions (Schipper et al., 2009).

(3) *HO-1, oxidative stress, iron deposition and mitochondrial damage*- In cultured astroglia, HO-1 up-regulation by transient transfection of the human *HMOX1* gene, or stimulation of endogenous HO-1 expression by exposure to β-amyloid, DA, hydrogen peroxide, TNF-α or IL-1β, promotes intracellular and intra-mitochondrial oxidative stress. The latter is evidenced, in part, by augmented levels of mitochondrial protein carbonyls (protein oxidation), 8-epiPGF2α (lipid peroxidation) and 8-OHdG (nucleic acid oxidation) and compensatory induction of the *Mnsod* gene relative to control preparations. The pro-oxidant effects of HO-1 in cultured astrocytes can be blocked by co-administration of natural antioxidants (ascorbate, melatonin, resveratrol), iron chelators (deferoxamine, phenanthroline) and the competitive inhibitor of HO activity, tin-mesoporphyrin (SnMP) (Schipper et al., 2009). Moreover, 3–6 days of exposure to the aforementioned stimuli or *HMOX1* transfection significantly augmented the incorporation of non-transferrin ^{59}Fe (or ^{55}Fe) into astroglial mitochondria without affecting transfer of the metal into whole-cell and lysosomal compartments, an effect not seen when the cultures were treated with SnMP or if diferric transferrin was used as the metal donor. The latter is commensurate with the view that the transferrin pathway plays little or no role in the pathological deposition of iron observed in AD- and PD-affected neural tissues (Schipper et al., 2009). In *HMOX1*-transfected astrocytes and cells exposed to DA, TNF-α or IL-1β, treatment with inhibitors of the mitochondrial permeability transition pore (cyclosporin A, trifluoperazine) interferes with mitochondrial iron trapping (Schipper et al., 2009). Conceivably, intracellular oxidative stress accruing from HO-1 activity promotes pore opening and flux of cytosolic iron into the mitochondrial matrix. The iron-laden mitochondria become distended and exhibit misshapen cristae and ruptured membranes.

The effete mitochondria merge with lysosomal constituents in a complex macroautophagic process, as determined by transmission electron microscopy and co-immunolocalization of mitochondrial epitopes with the lysosomal protease, cathepsin D (Schipper et al., 2009). These gliodystrophic changes recapitulate the 'core' pathology common to AD, PD and other aging-related neurodegenerative disorders as described above. Importantly, in co-culture paradigms, the HO-1-mediated gliopathy renders neuron-like PC12 cells prone to oxidative injury establishing a mechanism whereby primary insults to the astrocytic compartment may translate into neurological and behavioral dysfunction (Schipper et al., 2009).

To garner further support for this formulation, we recently generated GFAP.HMOX1 transgenic mice which selectively and conditionally express the human *HMOX1* gene in astrocytes. At 48 weeks of age following continuous induction of *HMOX1*, the mice exhibited all of the cytopathological features observed in HMOX1-transfected glial cultures, *viz.* iron deposition, oxidative mitochondrial damage, macroautophagy and corpora amylacea formation. Interestingly, the latter occurred along with subcortical neuritic degeneration, increased basal ganglia DA concentrations, robust hyperkinesia, behavioral stereotypy, and impaired prepulse inhibition to acoustic startle (Song et al., 2012b; Song et al., 2012a). A similar neuropathological profile was observed in older GFAP.HMOX1 mice which were manipulated to express the transgene between 8.5–19 months of age, although the phenotype was now one of hypodopaminergia and impaired motor coordination consistent with parkinsonism (Song et al., 2013).

As illustrated in **Figure 2,** our results indicate that astroglial HO-1 is well-poised to transduce a host of inimical influences/risk factors into 'core' neuropathology shared by many chronic CNS disorders. The model predicts further that a) the aberrant glial-neuronal interactions evoked are self-perpetuating and capable of driving the degenerative process after exposure to initiating stimuli may have subsided and b) identical sets of stressors may elicit diverse neurophenotypes commensurate with the anatomical localization, duration and intensity of the glial HO-1 response (Schipper et al., 2009; Song et al., 2012b).

(4) *HO-1 suppression in an AD mouse model*-OB-28 is an azole-based, brain-permeable and specific inhibitor of HO-1. Daily intraperitoneal injections of adult $APP_{swe}/PS1_{\Delta E9}$ transgenic mice, a well-characterized model of familial AD, with OB-28 (15 mg/kg) from 3 to 10 months of age significantly inhibited HO-1 activity in cerebral cortex and hippocampus with no overt adverse effects. The treated animals exhibited reduced astroglial activation (a measure of neuroinflammation) without any change in amyloid burden. Most importantly, $APP_{swe}/PS1_{\Delta E9}$ mice receiving OB-28 performed significantly better in a complex maze learning task relative to saline-treated controls (Gupta et al., 2014). These findings constitute first proof-of-principle that suppression of HO-1 activity ameliorates neuroinflammatory responses and cognitive deficits in a mouse model of AD independently of brain amyloid deposition.

Implications for neural repair: A priority issue in contemporary neuroscience concerns the degree to which indigent repair mechanisms and neuroplasticity may reverse CNS damage after offending stimuli have dissipated. Evidence of spontaneous lesion reversibility, neuroplasticity and neurological recovery has been documented in APP transgenic mouse models of AD (Kotilinek et al., 2002), mutant tau-driven models of AD and frontotemporal dementia (Sydow et al., 2011), the MPTP mouse model of PD (Schmidt and Ferger, 2001), rodent models of Huntington disease (Yamamoto et al., 2000) and several spinocerebellar degeneration mimics (Boy et al., 2009). These remarkable reports suggest that clinically-relevant neuroregeneration may be invoked in a broad spectrum of neurodegenerative disorders following interruption of the salient pathological pathways. In this article, we reviewed our position that (i) augmented iron deposition, oxidative stress, mitochondrial damage and macroautophagy constitute a single cohesive neuropathological 'lesion' capable of driving neurodegenerative processes in a host of chronic human CNS disorders, (ii) this neuropathological tetrad devolves from stressor-induced induction of the *HMOX1* gene in the astrocytic compartment and (iii) inhibition of glial HO-1 activity mitigates stress-related glial cytopathology in vitro and neurobehavioral deficits in at least one mouse model of AD. In this context, and based on the restorative

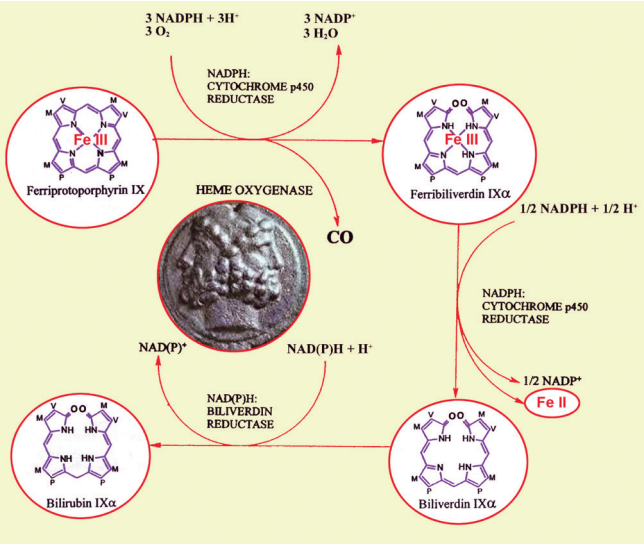

Figure 1 The heme catabolic pathway.
The heme degradation products, ferrous iron (Fe II), carbon monoxide (CO), and biliverdin/bilirubin may behave as either pro-oxidants or antioxidants accounting for the disparate influences of heme oxygenase expression on cell function and survival (symbolized by Janus faces). M: Methyl; V: vinyl; P: propionate [from Schipper et al. (2009) with permission].

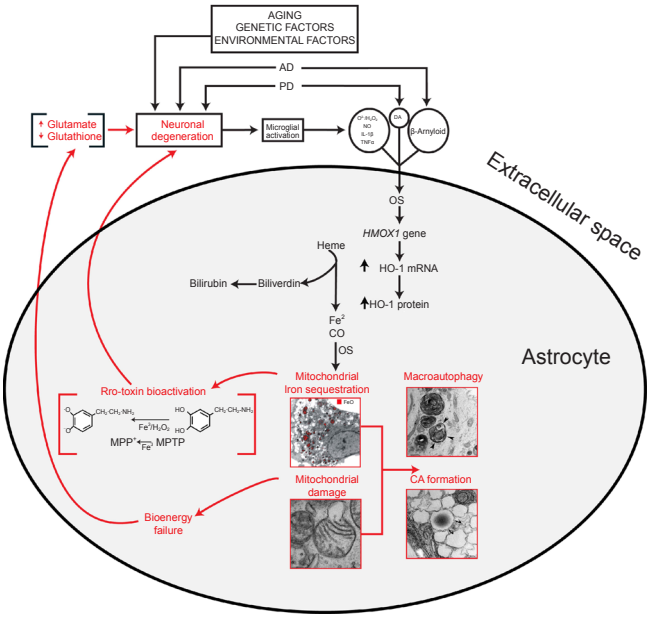

Figure 2 Putative roles of HO-1 in human neurodegenerative disorders.
Sustained or repeated up-regulation of HO-1 may exacerbate certain degenerative processes (red) characterized by mitochondrial damage and ferrous iron sequestration in astrocytes. The latter, in turn, may predispose to bioenergetic failure (ATP depletion), pro-toxin bioactivation, excitotoxicity, macroautophagy, and corpora amylacea formation. Neuronal degeneration stimulates microglial activation resulting in the release of ROS, NO, and proinflammatory cytokines (TNF-α, IL-1β). The latter may further induce *Hmox1* in indigent astroglia, completing a self-sustaining loop of pathological cellular interactions that may perpetuate oxidative damage and mitochondrial insufficiency within senescent and degenerating neural tissues. Genetic and environmental risk factors may confer disease specificity by superimposing unique pathological signatures on this core lesion. AD: Alzheimer's disease; CO: carbon monoxide; DA: dopamine; Fe2: ferrous iron; GSH: glutathione; HO-1: heme oxygenase-1; IL-1β: interleukin-1β; MPP+: methyl-4-phenylpyridinium; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NO: nitric oxide; OS: oxidative stress; PD: Parkinson's disease; TNF-α: tumor necrosis factor-α [Modified from Schipper et al. (2009) with permission].

properties of the degenerating CNS adduced from the afore cited animal models, we conjecture that glial HO-1 suppression may permit engagement of regenerative mechanisms instrumental for the repair of neural injury in AD, PD and related conditions. Further experimentation using the conditional GFAP.HMOX1 should disclose whether, or the extent to which, HO-1-mediated neuropathology is reversible.

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