

Heme Oxygenase-1: Transducer of Pathological Brain Iron Sequestration under Oxidative Stress

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ABSTRACT: Mechanisms responsible for the pathological deposition of redox-active brain iron in human neurological disorders remain incompletely understood. Heme oxygenase-1 (HO-1) is a 32-kDa stress protein that degrades heme to biliverdin, free iron, and carbon monoxide. In this chapter, we review evidence that (1) HO-1 is overexpressed in CNS tissues affected by Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), and other degenerative and nondegenerative CNS diseases; (2) the pro-oxidant effects of dopamine, hydrogen peroxide, β -amyloid, and proinflammatory cytokines stimulate HO-1 expression in some of these conditions; and (3) upregulation of HO-1 in astrocytes exacerbates intracellular oxidative stress and promotes sequestration of nontransferrin-derived iron by the mitochondrial compartment. A model is presented implicating glial HO-1 induction as a "final common pathway" leading to pathological iron sequestration and mitochondrial insufficiency in a host of human CNS disorders.

KEYWORDS: Alzheimer's disease; amyloid; astrocyte; cytokines; dopamine; heme oxygenase-1; iron; mitochondria; multiple sclerosis; oxidative stress; Parkinson's disease

IRON DEPOSITION AND THE CNS

Derangements in iron homeostasis and pathological deposition of this redox-active metal in brain have been implicated in a host of adult and pediatric conditions representing virtually every major category of neurological affliction: neurodegenerative (Alzheimer's disease, Parkinson's disease, progressive supranuclear palsy), metabolic (PANK-2 deficiency, aceruloplasminemia), immunologic (multiple sclerosis), ischemic (cerebral infarction), hemorrhagic (cerebral hematoma), traumatic (cerebral contusion), and infectious (HIV-1 encephalitis). Given iron's propensity to generate

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cytotoxic free radicals (Fenton chemistry), the advent of effective neuroprotection for many of these conditions may be facilitated by a thorough understanding of the pathophysiological mechanisms responsible for the abnormal brain metal profiles. While many of the mechanisms hypothesized will likely prove unique to specific disorders, others may constitute “final common pathways” shared by clusters of conditions that inevitably give rise to homologous patterns of aberrant brain metal deposition. In this chapter, evidence will be reviewed implicating induction of the enzyme, heme oxygenase-1, in astrocytes as one such common pathway to pathological brain iron sequestration and oxidative mitochondrial injury in Alzheimer’s disease (AD), Parkinson’s disease (PD), multiple sclerosis (MS), and other human neurological disorders. The model developed will also serve to unite several consistent neuropathological features of these conditions, namely, oxidative stress, iron mobilization, and mitochondrial insufficiency,¹ into a single “lesion” devolving on the action of HO-1. The primary literature attesting to central iron overload in these CNS disorders and other potential mechanisms mediating the abnormal accumulation of brain metals will be addressed elsewhere in this volume of the *Annals* and will not be covered here.

HEME OXYGENASE-1: REGULATION AND PHYSIOLOGY

Heme catabolism in a wide range of species is mediated by the heme oxygenase family of enzymes (E.C. 1:14:99:3; heme-hydrogen donor: oxygen oxidoreductase). Heme oxygenases are located within the endoplasmic reticulum, where they serve, in concert with NADPH cytochrome P450 reductase, to oxidize heme to biliverdin, free ferrous iron, and carbon monoxide (CO). Biliverdin is metabolized further to the bile pigment, bilirubin, by action of biliverdin reductase.² Mammalian cells express at least two isoforms of heme oxygenase, HO-1 (a.k.a. heat shock protein 32) and HO-2. A third protein, HO-3,³ may be a retrotransposition of the HO-2 gene unique to rats.⁴ HO-1 and HO-2 are encoded by distinct genes and exhibit significant differences with regard to molecular weight, electrophoretic mobility, susceptibility to proteolysis, tissue distribution, regulation, and antigenicity. Substrate and cofactor specificities are, however, identical for the two isoforms.⁵ Whereas HO-2 protein is widely distributed throughout the rodent neuraxis,⁶ basal HO-1 expression in the normal brain is confined to small groups of scattered neurons and neuroglia.⁷

In humans, the *ho-1* gene is located on chromosome 22q12 and contains 4 introns and 5 exons. A 500-bp promoter, a proximal enhancer, and 2 or more distal enhancers occur in the regulatory region of the mammalian *ho-1* gene.⁵ The latter exhibits AP-1, AP-2, nuclear factor kappa B (NFκB), and HIF-1 binding sites, as well as heat shock consensus (HSE) sequences, metal response elements (MtRE, CdRE), and antioxidant response elements (ARE). These response elements render the *ho-1* gene highly inducible by a wide array of pro-oxidant and inflammatory stimuli including heme, β-amyloid, dopamine, H₂O₂, UV light, transition metals, prostaglandins, Th1 cytokines, and lipopolysaccharide.^{5,8} Furthermore, susceptibility to transcriptional suppression by glucocorticoids is mediated by a 56-bp sequence (STAT-3 acute-phase response factor binding site) located within the *ho-1* promoter.⁹ A PEST [proline (P)–glutamic acid (E)–serine (S)–threonine (T)] sequence at the carboxy-terminus predisposes the HO-1 peptide to rapid degradation.¹⁰ In mammalian cells, the half-lives of HO-1 mRNA and protein are in the range of 3 h and 15–21 h, respectively.⁵

The products of the heme oxygenase reaction, biliverdin (and its derivative, bilirubin), iron, and CO are all biologically active molecules. In the face of oxidative challenge, induction of HO-1 may protect cells by augmenting the breakdown of pro-oxidant heme to the radical-scavenging bile pigments, biliverdin and bilirubin.^{7,11–14} Furthermore, biliverdin, bilirubin, and CO have been shown to have potent immunomodulatory properties. For example, in animal models, HO-1 upregulation attenuates inflammation of the cornea, pleura, and renal glomerulus; suppresses cardiac and renal xenograft rejection; and decreases mortality following systemic lipopolysaccharide exposure.^{15,16} In some tissues and under certain experimental conditions, the induction of HO-1 may actually promote rather than protect against cellular injury. The intracellular liberation of potentially cytotoxic free ferrous iron and CO may account for these “sinister” effects of HO-1. In many tissues, coinduction of ferritin provides a measure of cytoprotection by providing a “sink” for the excess intracellular iron. Under some circumstances, however, heme-derived iron and CO may exacerbate intracellular oxidative stress and cellular injury by promoting free radical generation within the mitochondrial compartment.^{17,18}

THE “JANUS” FACES OF HO-1 IN NEURAL INJURY

As described in the previous section, stressor-induced HO-1 expression may confer cytoprotection or, conversely, contribute to cellular injury. Perhaps nowhere has this disparate behavior of HO-1 engendered more controversy than in disorders of the nervous system. While there exists ample evidence for HO-1-mediated neuroprotection in various whole animal and tissue culture models of CNS injury and disease, a growing literature attesting to the neuroendangering aspects of HO-1 activity is also at hand. Details of this debate are beyond the scope of this article and are reviewed elsewhere.¹⁹ Suffice it to say that these conflicting positions should not be viewed as mutually exclusive; the intensity and chronicity of HO-1 induction and the chemistry of the local redox microenvironment may determine whether the anti-oxidant benefits of a diminished heme:bilirubin ratio or oxidative damage accruing from intracellular mobilization of iron/CO are predominant.^{20,21}

HO-1: TRANSDUCER OF MITOCHONDRIAL IRON TRAPPING IN “STRESSED” ASTROGLIA

In this section, we review evidence that HO-1 overexpression in astrocytes perturbs cellular redox homeostasis and promotes mitochondrial iron sequestration and oxidative injury. In the remainder of the paper, an argument will be developed implicating glial HO-1 expression as a common pathway leading to pathological iron deposition and oxidative mitochondrial damage in a host of human neurological disorders.

Stimulation of Glial HO-1 Expression and Mitochondrial Iron Sequestration

Cysteamine (CSH; 880 μ M), dopamine (DA; 1 μ M), tumor necrosis factor- α (TNF α ; 20 ng/mL), interleukin-1 (IL-1 β ; 20 ng/mL), and β -amyloid_{40/42} (3–15 μ M)

upregulate HO-1 mRNA, protein, and/or activity levels in cultured neonatal rat astroglia within 3–12 h of treatment. Within 3–6 days of exposure to these stimuli, sequestration of nontransferrin-derived ^{59}Fe (or ^{55}Fe) by the mitochondrial compartment is significantly augmented in these cells.^{22–24} These treatments had no appreciable effect on mitochondrial trapping of diferric transferrin-derived iron,^{22–24} an observation commensurate with the fact that brain iron deposition under various pathological conditions *in situ* may occur independently of transferrin and its receptor.⁸ The effects of the aforementioned stimuli on HO-1 expression and mitochondrial iron sequestration could be mimicked by hydrogen peroxide (300–500 μM) or menadione (100 μM) administration, and cotreatment with potent antioxidants (ascorbate, melatonin, or *trans*-resveratrol) attenuated the HO-1 response to CSH, DA, and the proinflammatory cytokines in these cells.^{22–24} Thus, oxidative stress is a likely common mechanism mediating glial *ho-1* gene induction in these experimental paradigms.

Pivotal Role of HO-1 in Mitochondrial Iron Trapping

Coadministration of tin mesoporphyrin (SnMP; 1 μM), a competitive inhibitor of heme oxygenase activity, or dexamethasone (DEX; 50 $\mu\text{g}/\text{mL}$), a transcriptional suppressor of the *ho-1* gene, significantly attenuated mitochondrial iron sequestration in cultured astrocytes exposed to DA, β -amyloid, $\text{TNF}\alpha$, or IL-1 β . Similarly, administration of SnMP or DEX abolished the pathological accumulation of mitochondrial ^{55}Fe observed in rat astroglia engineered to overexpress the human *ho-1* gene by transient transfection.^{22–24} These findings suggest that upregulation of HO-1 is a critical event in the cascade leading to excessive mitochondrial iron deposition in oxidatively challenged astroglia.

HO-1 Perpetuates Oxidative Stress in Cultured Astroglia

Treatment with ascorbate, melatonin, or resveratrol blocks the late, compensatory induction of the manganese superoxide dismutase gene in astrocytes transiently transfected with human HO-1 cDNA.¹⁸ In addition, preliminary data suggest that levels of protein carbonyls, isoprostanes, and 8-OHdG are significantly increased in mitochondrial fractions derived from astrocytes transfected with hHO-1 relative to sham-transfected cultures (author's unpublished results). Taken together, these findings strongly suggest that HO-1 overexpression, at least in astroglia, exacerbates intracellular oxidative stress. Treatment with cyclosporin A or trifluoperazine, potent inhibitors of the mitochondrial permeability transition pore, curtails mitochondrial iron trapping in hHO-1 transfected glia and cells exposed to DA, $\text{TNF}\alpha$, or IL-1 β .^{22,24} Conceivably, intracellular oxidative stress accruing from HO-1 activity promotes pore opening^{25,26} and influx of cytosolic iron to the mitochondrial matrix. The pathological accumulation of mitochondrial iron and other transition metals in astroglia overexpressing HO-1 may sensitize nearby neuronal elements to oxidative injury and thereby contribute directly to local neurodegenerative processes.^{27,28} As such, glial HO-1 activation may represent a legitimate target for pharmacological manipulation, bearing in mind the caveats implied above (see THE “JANUS” FACES OF HO-1 IN NEURAL INJURY). Further analysis of glial HO-1 as a potential therapeutic target is justified on account of its augmented expression in various human neurological disorders, as described in the following section.

HO-1 EXPRESSION IN HUMAN CNS DISORDERS

Alzheimer's Disease

In AD brain, HO-1 protein colocalizes to neurons, GFAP-positive astrocytes, ependymocytes, neurofibrillary tangles, senile plaques, corpora amylacea, and some vascular smooth muscle and endothelial cells.^{29,30} HO-1 immunoreactivity is greatly enhanced in neurons of the AD temporal cortex and hippocampus relative to corresponding tissues derived from nondemented controls matched for age and post-mortem interval.³⁰ An *in situ* hybridization study also revealed increased HO-1 mRNA levels in AD-affected brain tissues.³¹ We observed that 86% of GFAP-positive astrocytes residing within the AD hippocampus exhibited HO-1 immunoreactivity, whereas the fraction of hippocampal astroglia expressing HO-1 in normal control tissue was in the range of only 6–7%. Similarly, Western blots of protein extracts derived from AD hippocampus and temporal cortex revealed intense HO-1 bands, whereas the latter were faint or absent in normal control preparations.³⁰ As described above (see HO-1: TRANSDUCER OF MITOCHONDRIAL IRON TRAPPING IN “STRESSED” ASTROGLIA), oxidative stress resulting from excessive amyloid burden or the elaboration of proinflammatory cytokines may be responsible for the induction of HO-1 in the Alzheimer-diseased cerebral cortex and hippocampus. On the basis of the model presented earlier (see HO-1: TRANSDUCER OF MITOCHONDRIAL IRON TRAPPING IN “STRESSED” ASTROGLIA), we submit that the dysregulation of iron homeostasis and mitochondriopathy observed in AD brain^{1,30} may be a consequence of sustained HO-1 overproduction in the affected tissues.

A curious recent observation was the relative downregulation of HO-1 protein in AD choroid plexus epithelial cells.³² The latter may explain the apparent suppression of HO-1 concentrations previously documented in AD CSF relative to control values.³³ The subnormal levels of HO-1 protein in AD blood³³ and choroid plexus³² may be due to the presence of a circulating HO-1 suppressor factor in this disease that is currently under investigation.^{34,35}

Parkinson's Disease

In both PD and normal brain, moderate HO-1 immunoreactivity was observed in dopaminergic neurons of the substantia nigra.³⁶ In the PD samples, diseased dopaminergic neurons could be readily identified by the presence of cytoplasmic Lewy bodies that were prominently decorated with HO-1 staining.^{36,37} The proportion of GFAP-positive astroglia expressing HO-1 in the PD nigra was significantly greater (77.1%) than that computed in age-matched control subjects (18.7%). Percentages of GFAP-positive astroglia coexpressing HO-1 in other subcortical nuclei, such as the caudate, putamen, and globus pallidus, were relatively low and not substantially different between PD and control specimens.³⁶ Dopamine released from dying nigrostriatal neurons, dopamine-derived hydrogen peroxide (see HO-1: TRANSDUCER OF MITOCHONDRIAL IRON TRAPPING IN “STRESSED” ASTROGLIA), catechol-generated semiquinone radicals, and endogenous MPTP-like neurotoxins are all plausible inducers of astroglial HO-1 in the PD nigra.^{22,24} Augmentation of glial HO-1 activity may, in turn, promote the transferrin receptor-independent accumulation of iron and mitochondrial electron transport (complex I) deficits consistently reported in the basal ganglia of PD subjects.^{1,28}

Multiple Sclerosis

Proinflammatory cytokines, oxidative stress, and mitochondrial iron deposition have been implicated in the pathogenesis of MS and experimental autoimmune encephalomyelitis (EAE), an animal model of MS.^{24,38} In a neuropathological survey,²⁴ the percentage of GFAP-positive astrocytes expressing HO-1 in spinal cord plaques derived from MS patients (57.3%) was noted to be significantly greater than that observed in the spinal white matter of normal subjects (15.4%). In MS, glial HO-1 overexpression may be secondary to the enhanced release of TNF α , IL-1 β (see HO-1: TRANSDUCER OF MITOCHONDRIAL IRON TRAPPING IN “STRESSED” ASTROGLIA), or myelin basic protein³⁹ within the affected tissues. Upregulation of HO-1, in turn, may amplify intracellular oxidative stress and give rise to mitochondrial iron deposits reported in this condition (see HO-1: TRANSDUCER OF MITOCHONDRIAL IRON TRAPPING IN “STRESSED” ASTROGLIA).

Prominent HO-1 induction also occurs in the CNS of rodents with EAE (see chapter by S. LeVine in this volume). In a recent study, administration of tin-protoporphyrin-IX, a competitive inhibitor of heme oxygenase activity, ameliorated behavioral deficits, weight loss, and indices of neural oxidative stress in female SJL mice with EAE.⁴⁰ These findings conflict with an earlier report⁴¹ wherein worsening of disease was witnessed following metalloporphyrin suppression of heme oxygenase activity in Lewis rats with EAE. These interesting studies support an active role for HO-1 in the pathophysiology of EAE. They also signal further caution against facile extrapolation from one disease or disease model to another when contemplating manipulation of HO-1 as a therapeutic strategy for human neurological disorders.

Other CNS Disorders

Robust HO-1 overexpression has been documented in postmortem brain tissue procured from patients with cerebrovascular ischemia,⁴² progressive supranuclear palsy, Pick's disease, corticobasal ganglionic degeneration,⁴³ and cerebral malaria.⁴⁴ The pathological profiles of ischemic brain injury and many of the human neurodegenerations also feature abnormal iron mobilization, oxidative molecular damage, and bioenergetic failure¹ that we submit may be due, at least in part, to the antecedent induction of HO-1.

SUMMARY AND CONCLUSIONS

First, the *ho-1* gene is exquisitely sensitive to oxidative stress and, in astrocytes, is rapidly upregulated following exposure to the pro-oxidant effects of hydrogen peroxide, dopamine, β -amyloid, and Th1 cytokines. Free ferrous iron and CO derived from the HO-1-mediated degradation of heme exacerbates intracellular oxidative stress even after initiating insults may have dissipated. The intragial oxidative stress promotes opening of mitochondrial permeability transition pores, thereby allowing iron ions or low-molecular-weight iron chelates to accumulate within the mitochondrial matrix. With time, many of the effete, iron-laden mitochondria in these “stressed” astroglia undergo morphological transformation to Gomori-positive granules and corpora amylacea, inclusions that classically accumulate in aging and degenerating neural tissues⁴⁵ (FIG. 1). In spite of these profound changes, the astro-

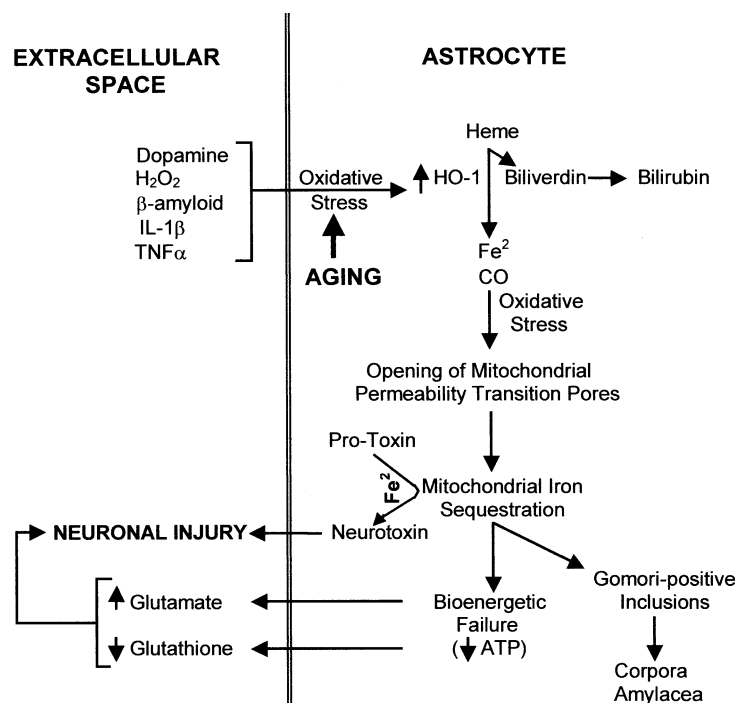


FIGURE 1. Putative role of astroglial HO-1 in pathological iron deposition and mitochondrial insufficiency in human neurodegenerative and inflammatory disorders.

cytes tend to remain viable because (i) they are endowed with potent antioxidant defenses in comparison with other neural cell types, (ii) they rapidly elaborate a number of heat shock proteins known to confer cytoprotection, and (iii) in contradistinction to neurons and oligodendroglia, their capacity to shift to robust anaerobic metabolism may allow them to sacrifice a significant proportion of their mitochondria with relative impunity.²²

Second, prolonged survival of iron-laden astrocytes *in situ* may have important ramifications for the pathological and clinical progression of neurodegenerative diseases. Using electron spin resonance spectroscopy, we demonstrated that the glial mitochondrial iron behaves as a pseudo-peroxidase activity that promotes the oxidation of dopamine and other catechols to potentially neurotoxic ortho-semiquinone radicals.²⁸ The redox-active glial iron also facilitates bioactivation of the protoxin, MPTP, to the dopaminergic neurotoxin, MPP+, in the face of monoamine oxidase blockade.⁴⁶ We observed that neuronlike PC12 cells grown on a substratum of astrocytes replete with mitochondrial iron are far more susceptible to dopamine/H₂O₂-related killing than PC12 cells cocultured with control, "iron-poor" astroglia.²⁷ Taken together, these findings suggest that the progressive increase in glial mitochondrial iron that has been documented in subcortical regions of the aging rodent and human brain may enhance the susceptibility of the latter to parkinsonism and other free radical-related neurodegenerative disorders²⁸ (FIG. 1).

Third and last, HO-1 protein is overexpressed in astrocytes and other cells indigenous to CNS tissues affected by AD, PD, other aging-related neurodegenerations, MS, ischemia, and malaria. Numbers of neuroglia immunoreactive for HO-1 also correlate positively with aging in the normal human cerebral cortex and hippocampus.⁴⁷ These observations, in conjunction with the aforementioned *in vitro* findings, suggest that the aberrant deposition of (nontransferrin) iron, redox pathophysiology, and mitochondrial insufficiency characterizing many, if not all, of these disparate conditions may represent immediate downstream effects of glial HO-1 induction in the afflicted tissues (FIG. 1). This is not to imply that one should immediately move to suppress brain HO-1 in these patients; as discussed above, HO-1 is a “double-edged sword” that, under certain circumstances, may confer neuroprotection. Further investigation will be required to resolve these important issues. It may also prove interesting to see whether HO-1 contributes to pathological brain iron deposition in subjects with PANK-2 deficiency (formerly Hallervorden-Spatz disease), aceruloplasminemia, Friedreich’s ataxia, sideroblastic anemia with ataxia, and neuroferritinopathy above and beyond the dysregulation of metal homeostasis incurred by the primary genetic defects.

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REFERENCES

1. BEAL, M.F. 1996. Mitochondria, free radicals, and neurodegeneration. *Curr. Opin. Neurobiol.* **6**: 661–666.
2. MAINES, M.D. 2000. The heme oxygenase system and its functions in the brain. *Cell. Mol. Biol. (Noisy-Le-Grand)* **46**: 573–585.
3. MCCOUBREY, W.K., JR., T.J. HUANG & M.D. MAINES. 1997. Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. *Eur. J. Biochem.* **247**: 725–732.
4. SCAPAGNINI, G. *et al.* 2002. Gene expression profiles of heme oxygenase isoforms in the rat brain. *Brain Res.* **954**: 51–59.
5. DENNERY, P.A. 2000. Regulation and role of heme oxygenase in oxidative injury. *Curr. Top. Cell. Regul.* **36**: 181–199.
6. VERMA, A. *et al.* 1993. Carbon monoxide: a putative neural messenger. *Science* **259**: 381–384.
7. BARANANO, D.E. & S.H. SNYDER. 2001. Neural roles for heme oxygenase: contrasts to nitric oxide synthase. *Proc. Natl. Acad. Sci. USA* **98**: 10996–11002.
8. SCHIPPER, H.M. 2000. Heme oxygenase-1: role in brain aging and neurodegeneration. *Exp. Gerontol.* **35**: 821–830.
9. LAVROVSKY, Y., G.S. DRUMMOND & N.G. ABRAHAM. 1996. Downregulation of the human heme oxygenase gene by glucocorticoids and identification of 56b regulatory elements. *Biochem. Biophys. Res. Commun.* **218**: 759–765.
10. DWYER, B.E. *et al.* 1992. Heme oxygenase is a heat shock protein and PEST protein in rat astroglial cells. *Glia* **5**: 300–305.
11. STOCKER, R. *et al.* 1987. Bilirubin is an antioxidant of possible physiological importance. *Science* **235**: 1043–1046.

12. NAKAGAMI, T. *et al.* 1993. A beneficial role of bile pigments as an endogenous tissue protector: anti-complement effects of biliverdin and conjugated bilirubin. *Biochim. Biophys. Acta* **1158**: 189–193.
13. LLESUY, S.F. & M.L. TOMARO. 1994. Heme oxygenase and oxidative stress: evidence of involvement of bilirubin as physiological protector against oxidative damage. *Biochim. Biophys. Acta* **1223**: 9–14.
14. DORE, S. *et al.* 1999. Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury. *Proc. Natl. Acad. Sci. USA* **96**: 2445–2450.
15. WILLIS, D. *et al.* 1996. Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat. Med.* **2**: 87–90.
16. TOSAKI, A. & D.K. DAS. 2002. The role of heme oxygenase signaling in various disorders. *Mol. Cell. Biochem.* **232**: 149–157.
17. ZHANG, J. & C.A. PIANTADOSI. 1992. Mitochondrial oxidative stress after carbon monoxide hypoxia in the rat brain. *J. Clin. Invest.* **90**: 1193–1199.
18. FRANKEL, D., K. MEHINDATE & H.M. SCHIPPER. 2000. Role of heme oxygenase-1 in the regulation of manganese superoxide dismutase gene expression in oxidatively-challenged astroglia. *J. Cell. Physiol.* **185**: 80–86.
19. MAWAL, Y., B.D. KRAVITZ & H.M. SCHIPPER. 2002. Heme oxygenase-1 and Alzheimer disease. *In* *Heme Oxygenase in Biology and Medicine*, pp. 145–155. Kluwer Academic/Plenum. New York.
20. GALBRAITH, R. 1999. Heme oxygenase: who needs it? *Proc. Soc. Exp. Biol. Med.* **222**: 299–305.
21. SUTTNER, D.M. & P.A. DENNERY. 1999. Reversal of HO-1 related cytoprotection with increased expression is due to reactive iron. *FASEB J.* **13**: 1800–1809.
22. SCHIPPER, H.M. 1999. Glial HO-1 expression, iron deposition, and oxidative stress in neurodegenerative diseases. *Neurotox. Res.* **1**: 57–70.
23. HAM, D. & H.M. SCHIPPER. 2000. Heme oxygenase-1 induction and mitochondrial iron sequestration in astroglia exposed to amyloid peptides. *Cell. Mol. Biol. (Noisy-Le-Grand)* **46**: 587–596.
24. MEHINDATE, K. *et al.* 2001. Proinflammatory cytokines promote glial heme oxygenase-1 expression and mitochondrial iron deposition: implications for multiple sclerosis. *J. Neurochem.* **77**: 1386–1395.
25. PETRONILLI, V. *et al.* 1993. Physiological effectors modify voltage sensing by the cyclosporin A-sensitive permeability transition pore of mitochondria. *J. Biol. Chem.* **268**: 21939–21945.
26. BERNARDI, P. 1996. The permeability transition pore: control points of a cyclosporin A-sensitive mitochondrial channel involved in cell death. *Biochim. Biophys. Acta* **1275**: 5–9.
27. FRANKEL, D. & H.M. SCHIPPER. 1999. Cysteamine pretreatment of the astroglial substratum (mitochondrial iron sequestration) enhances PC12 cell vulnerability to oxidative injury. *Exp. Neurol.* **160**: 376–385.
28. SCHIPPER, H.M. 2001. Mitochondrial iron deposition in aging astroglia: mechanisms and disease implications. *In* *Mitochondrial Ubiquinone (Coenzyme Q): Biochemical, Functional, Medical, and Therapeutic Aspects in Human Health and Disease*, pp. 267–280. Prominent Press. Scottsdale, AZ.
29. SMITH, M.A. *et al.* 1994. Heme oxygenase-1 is associated with the neurofibrillary pathology of Alzheimer's disease. *Am. J. Pathol.* **145**: 42–47.
30. SCHIPPER, H.M., S. Cisse & E.G. STOPA. 1995. Expression of heme oxygenase-1 in the senescent and Alzheimer-diseased brain. *Ann. Neurol.* **37**: 758–768.
31. PREM Kumar, D.R. *et al.* 1995. Induction of heme oxygenase-1 mRNA and protein in neocortex and cerebral vessels in Alzheimer's disease. *J. Neurochem.* **65**: 1399–1402.
32. ANTHONY, S.G. *et al.* 2003. Stress protein expression in the Alzheimer-diseased choroid plexus. *J. Alzheimer's Dis.* **5**: 171–177.
33. SCHIPPER, H.M. *et al.* 2000. Evaluation of heme oxygenase-1 as a systemic biological marker of sporadic AD. *Neurology* **54**: 1297–1304.
34. BERLIN, D., A. LIBERMAN & H.M. SCHIPPER. 2002. Partial characterization of a heme oxygenase-1 suppressor in Alzheimer plasma. *Ann. Neurol.* **52**(suppl. 1): S30.

35. KRAVITZ, S. *et al.* 2002. Heme oxygenase-1 suppressor activity in Alzheimer plasma. *Ann. Neurol.* **52**(suppl. 1): S31.
36. SCHIPPER, H.M., A. LIBERMAN & E.G. STOPA. 1998. Neural heme oxygenase-1 expression in idiopathic Parkinson's disease. *Exp. Neurol.* **150**: 60–68.
37. CASTELLANI, R. *et al.* 1996. Glycooxidation and oxidative stress in Parkinson disease and diffuse Lewy body disease. *Brain Res.* **737**: 195–200.
38. LEVINE, S.M. 1997. Iron deposits in multiple sclerosis and Alzheimer's disease brains. *Brain Res.* **760**: 298–303.
39. BUSINARO, R. *et al.* 2002. Myelin basic protein induces heme oxygenase-1 in human astroglial cells. *Glia* **37**: 83–88.
40. CHAKRABARTY, A., M.R. EMERSON & S.M. LEVINE. 2003. Heme oxygenase-1 in SJL mice with experimental allergic encephalomyelitis. *Multiple Sclerosis* **9**: 372–381.
41. LIU, Y. *et al.* 2001. Heme oxygenase-1 plays an important protective role in experimental autoimmune encephalomyelitis. *Neuroreport* **12**: 1841–1845.
42. BESCHORNER, R. *et al.* 2000. Long-term expression of heme oxygenase-1 (HO-1, HSP-32) following focal cerebral infarctions and traumatic brain injury in humans. *Acta Neuropathol. (Berlin)* **100**: 377–384.
43. CASTELLANI, R. *et al.* 1995. Evidence for oxidative stress in Pick disease and cortico-basal degeneration. *Brain Res.* **696**: 268–271.
44. SCHLUESENER, H.J. & K. SEID. 2000. Heme oxygenase-1 in lesions of rat experimental autoimmune encephalomyelitis and neuritis. *J. Neuroimmunol.* **110**: 114–120.
45. MYDLARSKI, M.B. 1998. Astrocyte granulogenesis and the cellular stress response. *In* *Astrocytes in Brain Aging and Neurodegeneration*, pp. 207–234. R. G. Landes. Austin, TX.
46. DI MONTE, D.A. *et al.* 1995. Iron-mediated bioactivation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in glial cultures. *Glia* **15**: 203–206.
47. HIROSE, W., K. IKEMATSU & R. TSUDA. 2003. Age-associated increases in heme oxygenase-1 and ferritin immunoreactivity in the autopsied brain. *Leg. Med. (Tokyo)* **5**(suppl.): S360–S366.