HEME DEGRADATION IN ACUTE BRAIN INJURY: BENEFICIAL OR DETRIMENTAL TO RECOVERY?

*Tiago Prazeres Moreira; M.D., PhD**

ABSTRACT

The mechanisms of heme degradation have re-emerged in the past decade to become a debated topic in the study of recovery from brain injury caused by ischemia, trauma or hemorrhage. The major players of heme degradation in response to acute brain injury are the heme oxygenases 1 and 2 (HO-1, HO-2) and biliverdin reductase (BVR). The generation of free iron, carbon monoxide and biliverdin/bilirubin upon heme degradation has been shown to induce a variety of molecular, cellular and vascular effects which, at present, remain contradictory. Experimental and clinical evidence of neuroprotective actions of the heme oxygenases in acute brain injury has been denied by cytotoxic effects found in neurodegenerative diseases or by recent reports on the detrimental effects of bilirubin oxidation products (BOXes). In general, induction of HO-1 upon acute brain injury seems to be neuroprotective, provided that hemorrhage is absent from the clinical manifestations of the primary pathophysiological event.

1. INTRODUCTION

Heme is the main constituent of a variety of hemoproteins such as hemoglobin, myoglobin, cytochromes, guanyl cyclase and nitric oxide synthase. During episodes of ischemia/hemorrhage, edema or trauma to the brain, heme is released by breakdown of blood hemoglobin and other hemoproteins in the cytoplasm, and/or is released from mitochondrial cytochromes of neurons and glia[1-6]. The heme molecule is subsequently degraded by enzymatic and non-enzymatic mechanisms, both requiring a reducing agent for the activation of O_2 and reduction of oxidated iron (Fe^{3+}) to reduced iron (Fe^{2+}). The enzymatic degradation of heme is catalysed by the ***heme oxygenases*** in the presence of NADPH[7-9], which leads to the for-

* Institut de Physiologie, Faculté de Biologie et Médecine, Université de Lausanne (UNIL), Lausanne, Switzerland

Address for correspondence:
Dr. Tiago Moreira, Département de Physiologie, Université de Lausanne,
Rue du Bugnon 7-Annexe, 2éme étage, CH-1005 Lausanne, Switzerland,
Tel.: Int-41-21-692 55 47; Fax: Int-41-21-692 55 05
E-mail: tiago.more@gmail.com

mation of equimolar amounts of carbon monoxide (CO), Fe^{2+} and the α-isomer of biliverdin (Figure 1).

CO induces vasodilation by relaxation of vascular smooth muscle cells at physiologic concentrations[11,12] and regulates the activity of downstream effectors such as soluble guanyl cyclase, the nitric oxide synthases and cyclooxygenases 1 and 2[13]. Biliverdin is immediately converted into bilirubin by biliverdin reductase, an enzyme present in excess in virtually all tissues. A decreased heme-to-bilirubin ratio is beneficial since a reduction in heme availability will decrease the pro-oxidant effect of heme and increase the formation of bilirubin, which has been shown to have a neuroprotective effect at physiological levels (see further in *biliverdin reductase*). As in other tissues, iron can rapidly be sequestered by iron-binding proteins like ferritin, transferrin and ceruloplasmin[14,15]. However, free iron (Fe^{2+}) derived from the degradation of hemoproteins can be harmful to cells either by reacting with H_2O_2 to form the hydroxyl radical $HO^{\bullet}$ (Fenton's reaction) or by causing lipid peroxidation in cellular membranes to produce alkoxy and peroxy radicals, unless it is oxidized by ceruloplasmin and bound to transferrin[14,16].

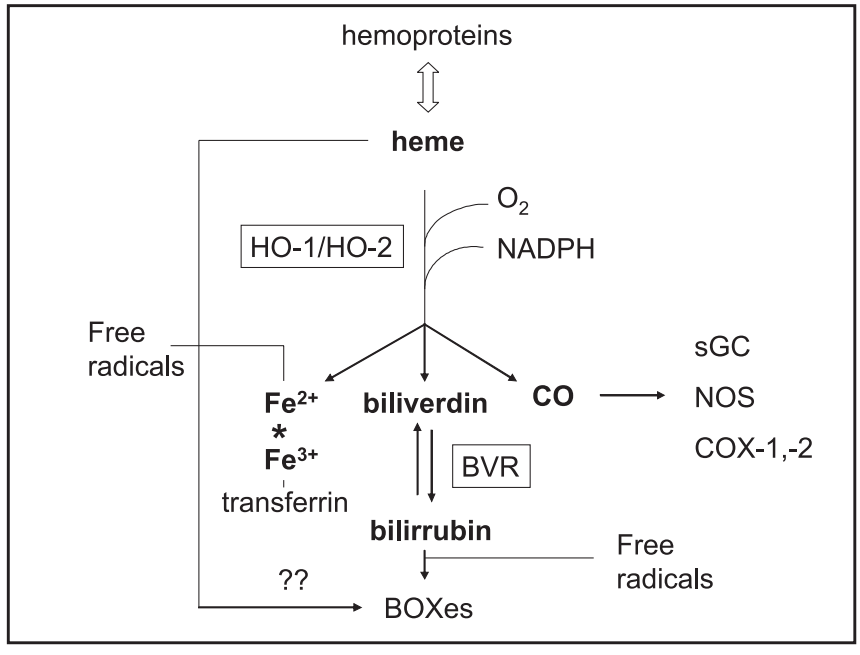

Figure 1. Degradation of heme and other hemoproteins by the heme oxygenases system, a reaction requiring O_2 and NADPH. Legend: HO – heme oxygenase, BVR – biliverdin reductase, CO – carbon monoxide, sGC – soluble guanyl cyclase, NOS – nitric oxide synthases, COX – cyclooxygenase, * – Fenton's reaction or oxidation by ceruloplasmin generates oxidated iron (Fe^{3+}). The products of heme degradation, reduced iron, CO and biliverdin have biological effects that are dependent on the concentration and local environment. Biliverdin is further converted to bilirubin by BVR via redox cycling. The excess availability of BVR balances the tissue concentrations of bilirubin and biliverdin, reported to be cytoprotective under physiologic concentrations. More recently, bilirubin was shown to be oxidized by free radical action originating three products: BOX A, BOX B and MVM (4-methyl-3-vinylmaleimide) but it is still unclear if heme can be directly oxidized by free radical action to generate similar molecules[10].

2. THE HEME OXYGENASES

Two major isozymes of heme oxygenase (HO-1, HO-2) were identified in the endoplasmic reticulum of mammals[13,17,18]. The two isoforms share the same mechanisms of heme catalysis, substrate specificity, co-factor requirements and cleave the heme molecule at the α-meso carbon bridge. Heme oxygenase –1 is a heat-shock protein (HSP, also referred to as HSP-32) induced by heme from aging red blood cells, mitochondrial heme-containing proteins and various sources of cellular stress[19-23]. A third isozyme, HO-3, was recently reported [24] but appears to be a non-functional derivative of HO-2[25,26]. HO-1 is the smaller molecule of three, with 288 a.a., m.w. of 30.000 to 33.000 Da[17], while HO-2 has 316 a.a., m.w. of 36.000 Da[18]. HO-1 and HO-2 share 50% similarity in nucleotide composition and 43% in a.a. sequences. The HO-1 gene is highly inducible by pro-oxidant and inflammatory stimuli such as β-amyloid, dopamine, H_2O_2, prostaglandins, kainic acid, ultraviolet light, Th1 cytokines, lipopolysaccharide, among other[27-29]. The promotor region of HO-1 contains several binding sites for regulatory factors

such as heat-shock factor, AP-1, NF-κB and metal regulatory elements[30-32]. The induction of HO-1 is regulated by the nuclear factor E2-related transcription factor (Nrf 2), which translocates to the nucleus and binds to the antioxidant response element (ARE) in the promoter region of the HO-1 gene, in response to the various HO-1 inducers. The promotor region of the HO-2 gene only contains a binding site for the glucocorticoid response element[33-35]. Phosphorylation of HO-2 by protein kinase C and phorbol esters lead to increased HO-2 catalytic activity and subsequent bilirubin production[36].

2.1. The role of heme oxygenases in the brain

Among the heme-oxygenases, HO-2 is the main isozyme expressed in the adult rat brain and is responsible for most of the heme-degrading activity in the brain[37-39]. Both HO-1 and -2 are expressed in neurons, with HO-1 displaying a restricted distribution across the brain, the highest expression levels found in the DG and ventromedial hypothalamus[40]. On the contrary, HO-2 is widely expressed in neurons of the forebrain, midbrain, hippocampus (pyramidal cells) and dentate gyrus (granule cells), basal ganglia, thalamus, mitral cells of the olfactory bulb, cerebellum and brainstem[33,40-42]. Despite the lower number of brain areas of HO-1 neuronal expression, this enzyme is further expressed in glial cells (astrocytes and microglia). Increased HO-1 expression in astroglia is considered to be neuroprotective against increased oxidative stress[21,43,44]. In this regard, expression of HO-1 in neurons is not responsive to oxidative stress unlike HO-1 induction in glial and astrocytic cells[44-48]. HO-1 was postulated to have antioxidant or cytotoxic effects depending on the intensity and chronicity of HO-1 induction and local, cellular redox conditions[49,50]. Degradation

	HO-1	HO-2
Cell type	Neurons Astrocytes Microglia/macrophages	Neurons
Brain areas	Hippocampus (dentate gyrus) Ventromedial and paraventricular nuclei of the hypothalamus Thalamus Olfactory bulb Cerebellum Brainstem	Forebrain and midbrain Hippocampus (dentate gyrus) Basal ganglia
Inducing stimuli	β-amyloid DopamineH_2O_2, other free radicals Prostaglandins Kainic acid UV light	Glucocorticoids

Table 1. Differential expression of HO-1 and HO-2 in different cell types and brain areas. HO-1 is expressed at low levels in the brain whereas HO-2 is responsible for most of the heme oxygenase activity under basal conditions. On the contrary, the HO-1 isozyme is responsive to a broader variety of stimuli.

of heme may also enhance blood-brain barrier disruption since red blood cell lysis and edema formation have been linked to HO-1 overexpression and iron accumulation[51-54].

Under basal conditions, activated microglia were shown to contain elevated levels of ferritin which bind iron (Fe^{3+}) released during HO activity when degrading hemoproteins contained in the engulfed debris[55]. The fact that microglia contain higher levels of ferritin when compared to neurons, renders the latter more vulnerable to increased iron-load derived from HO-1 activity[55]. Several lines of evidence coming from experimental animal models and human brain injury studies show that HO-1 is highly induced after injury while HO-2 levels remain relatively unchanged. Thus, different patterns of HO-1 expression can be found in astrocytes, activated microglia or neurons, depending on the experimental model.

2.2. Studies in animal models of brain injury

Following focal brain ischemia in the Wistar rat, HO-1 immunoreactivity was mostly detected in neurons and astrocytes[22,56]. However, other studies reported ischemia-induced HO-1 expression in astrocytes and microglia[57] or mainly in microglia[58]. After traumatic brain injury in Sprague-Dawley rats, HO-1 was mainly induced in astrocytes[21], but also on microglia[59] or both[60]. In Wistar rats, HO-1 expression induced by traumatic brain injury (TBI) was detected in both glial cell types[61]. HO-1 expression thus seems to be induced mainly in astroglia following acute brain injury.

2.3. Studies in neuronal and astrocytic cell cultures

In cultured astrocytes from the rat brain, HO-1 upregulation was shown to promote mitochondrial sequestration of non-transferrin-derived iron, to provoke oxidative damage to mitochondrial lipids, proteins, nucleic acids, and to enhance cell death and growth arrest[62]. Other studies however, contradicted these findings. Indeed, HO-1 was reported to protect cultured cortical astrocytes from oxidative stress resulting from exposure to hemoglobin and H_2O_2[44,63]. HO-1 overexpression was also reported to attenuate glucose-mediated oxidative stress in endothelial cell cultures [64]. Recently, HO-1 induction was found to protect astrocytes from exposure to hemoglobin-induced oxidative injury, whereas neurons showed increased cell death when exposed to hemoglobin, indicating that neurons may be more vulnerable to heme degradation[65].

Some *in vitro* studies disputed a protective role of HO-2 by showing that gene deletion of this isozyme attenuates hemin- and hemoglobin-mediated oxidative stress in murine cortical neurons and provokes extensive cell death in astrocytic cultures[66,67].

2.4. Studies in HO-1 and HO-2 transgenic mice

The HO-1 knockout mice (-/-) are born debilitated and die within 3-4 months of birth with massive iron deposition in the liver and other tissues. Since this accumulation of iron occurred without elevation of circu-

lating iron in the blood, it was suggested that the cytoprotective effect of HO-1 may rely on the efflux of iron from the tissues into the circulation. Experimental middle cerebral artery-occlusion (MCA-O) in these animals did not provoke brain infarts larger than those observed in control mice[68].

Studies in HO-2 knockout (-/-) mice suggest that the HO-2 isozyme is necessary for the recovery of TBI[69]. These animals showed delayed motor recovery as well as evidence of increased lipid peroxidation following TBI[39]. Another study in the HO-2 knockout mouse also revealed increased neuronal death following focal ischemia caused by MCA-O[69]. Furthermore, cultured neurons from HO-2 knockout (-/-) mice show increased apoptotic cell death upon oxidative stress challenge[36].

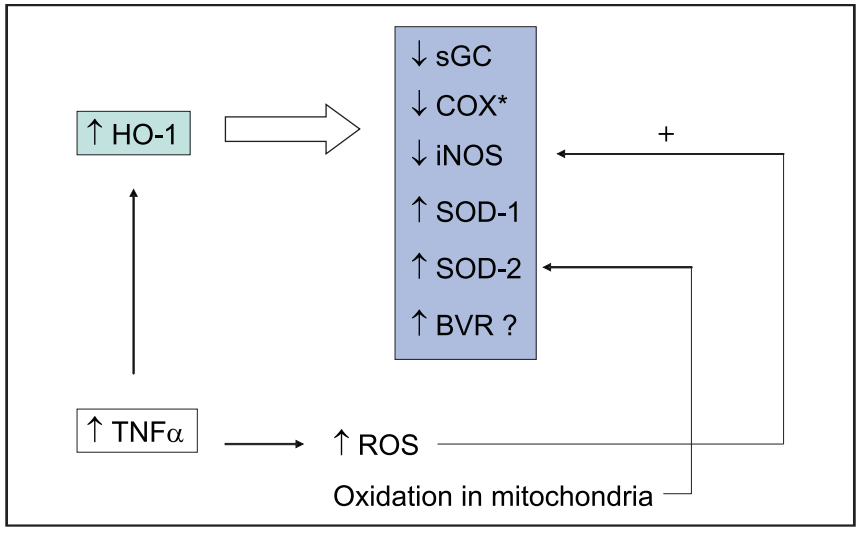

Figure 2. Actions of increased HO-1 expression in a variety of enzymes induced by oxidative and cellular injury described in the literature. Legend: HO-1 – heme oxygenase 1, sGC – soluble guanyl cyclase, COX – cyclooxygenases, * – to date, only shown in endothelial cells[79,80], iNOS – inducible nitric oxide synthase, SOD – superoxide dismutase, BVR – biliverdin reductase, ROS – reactive oxygen species, TNFα – tumor necrosis factor alpha. Increases in BVR due to HO-1 overexpression are expected but since BVR exists in excess in all tissues it is still unclear the exact the degree of HO-1 overexpression that can trigger BVR overexpression. TNFα was shown to stimulate HO-1 expression and to co-localize with this enzyme in receptor mediated apoptosis.

In mice overexpressing HO-2 (+/+), apoptotic cell-death was reduced in cortical, hippocampal and cerebellar cell cultures[36,70,71]. These rats show a modest (115% of control) increase in HO-1 mRNA expression in the brain, with higher heme-degrading activity (around 150% of control activity in non-transgenic mice) and pronounced HO-1 staining in the pyramidal layers of the CA1, CA3, DG and hilus[72].

2.5. Further interactions of heme-oxygenase-1

HO-1 was shown to stimulate SOD-1 activity and to decrease $O_2^{\bullet -}$ concentrations[73]. Increased HO-1 expression was also reported to induce SOD-2 expression, promoting cytoprotective effects[74,75]. SOD-2 was also identified as a downstream effector of HO-1 in astrocytes during nitrosative stress[76]. Moreover, HO-1 inhibition by zinc protoporphyrin IX has been shown to reduce SOD-2 expression[76]. The increase in SOD-2 following HO-1 activation is supposed to be compensatory because free-ferrous iron can exert a pro-oxidant effect on the mitochondrial compartment[77]. Enzymes such as soluble guanyl cyclase, cyclooxygenase and nitric oxide synthase require heme for their functions, and their activity is thus reduced by HO-1 upregulation. In addition, COX-1 was shown to accumulate in activated microglia/macrophages during recovery from brain ischemia or trauma[78]. Some of the possible interactions of HO-1 with other antioxidant and pro-inflammatory, pro-apoptotic enzymes and peptides are summarized in Figure 2.

3. BILIVERDIN REDUCTASE

Biliverdin is formed by the oxidative cleavage of the heme molecule at the α-meso carbon bridge by the heme oxygenase system[7-9]. Biliverdin is then converted to bilirubin by biliverdin reductase (BVR), a zinc metalloprotein, coupled with the oxidation of NADH and NADPH, its co-factors[81-83]. BVR can yield bilirubin at two different values of pH: in acidic pH (6.7) NADH is the used co-factor, in basic pH (8.7) NADPH is the used co-factor. In total, four isomers of biliverdin can be formed from heme also through non-enzymatic reactions, the most abundant being the α-isomer[84]. Each molecule of bilirubin is reconverted to biliverdin through oxidation by biliverdin reductase. BVR exists in excess in all tissues including the brain[85-87] and each molecule of bilirubin is rapidly oxidized into biliverdin. The high level of BVR immediately reduces biliverdin back to bilirubin (redox cycling), which prevents biliverdin to accumulate at detectable levels[88].

Since the early 1950's, when the link between severe unconjugated hyperbilirubinemia and neurologic dysfunction in the newborn was scientifically proven[89], that bilirubin has been regarded as a potential threat to the CNS by clinicians. Bilirubin encephalopathy or *kernicterus* is characterized by atethoid cerebral palsy, impaired upward gaze and deafness. However, recent studies of bilirubin metabolism in the brain have shown that this pigment has a wide array of neuroprotective actions at physiological levels. The neuroprotective action of bilirubin was proposed to occur via redox cycling of bilirubin, which in turn scavenges reactive oxygen species. The lack of BVR has been shown to increase cellular vulnerability to oxidative stress [88]. The neuroprotective effects of bilirubin are seen at low concentrations (about 10 nM), that is, at the normal endogenous levels in the brain, while *kernicterus* is associated with a 1000-fold increase in bilirubin concentrations.

3.1. Studies in astrocytic and microglial cell cultures

Treatment of astrocyte cultures with 50 μM of unconjugated bilirubin (equivalent to 148 nM of free unconjugated bilirubin) was shown to trigger the release of TNFα, IL1-β and IL-6[90] and to activate the MAPK and NF-κB signalling pathways, leading to an increase in astrocyte death[91]. Similarly, treatment of microglial cultures with 50 or 100 μM of unconjugated bilirubin was found to induce microglia activation, stimulate TNFα, IL1-β, IL-6 and glutamate release, and to increase microglial cell death[92].

At present, however, it is still unclear the exact concentration at which bilirubin becomes toxic. A growing body of evidence shows that not only bilirubin but also biliverdin have potent antioxidant properties[93,94], as well as immunomodulatory (e.g. blocking IL-2 production) and anti-complement actions[94-98]. Intracellular bilirubin is capable of inhibiting protein kinase C, c-AMP dependent protein kinases, NADPH oxidase and protein phosphorylation[99,100]. Physiological concentrations of bilirubin were shown to decrease the expression of pro-inflammatory genes such as

monocyte chemotactic protein-1 (MCP-1), vascular cell adhesion molecule -1 (VCAM-1) and macrophage colony stimulating factor (MCSF) in cultured human aortic endothelial cells and improved endothelium-dependent vascular relaxation[101]. Bilirubin is also a potent inhibitor of monocyte adhesion to the vascular endothelium and of chemotaxis[102,103].

3.2. Studies in animal models of brain injury

In the rat brain, BVR was suggested to participate in the first line of defense against ischemic injury due to its antioxidant properties against free radical attack on cellular membranes[11,94,104,105]. For instance, MCA-O in the mouse induced BVR mRNA and protein expression within the peri-ischemic cortical areas, namely in surviving neurons in cortical layers III and V and in the caudate nucleus[106]. Furthermore, BVR expression was found to be induced by hyperthermia[86] while bilirubin was capable of counteracting oxidative damage in models of autoimmune encephalomyelitis and to inhibit iNOS expression and NO production in lipopolysaccharide-treated macrophages[107,108].

3.3. Bilirubin oxidation products (BOXes)

Bilirubin metabolites provoked by free radical oxidation were recently described[10]. The oxidation of bilirubin at the two ends of the molecule originates the isomers BOX A and BOX B, as well as MVM (4-methyl-3-vinylmaleimide), a previously isolated product of biliverdin[109]. Oxidation of bilirubin with hydrogen peroxide was shown to produce vasoactive compounds that increase oxygen consumption and contractility of porcine carotid artery rings[10]. At present, it is not yet known if direct oxidation of heme within hemoglobin leads to the formation of BOXes or similar molecules (see Figure 1). The direct application of BOXes in the exposed cortex of rats produced a dose-dependent vasospasm in dural and cerebral vessels lasting up to 24 h, in association with increased HO-1 gene expression in the subcortical white matter[110].

4. CLINICAL FINDINGS

In the clinical setting, focal brain ischemia was reported to induce more pronounced peri-lesional HO-1 expression in astrocytes and weaker HO-1 microglial expression within the first 24 h post-infarction[111]. Microglial expression of HO-1 was more commonly associated with traumatic brain injury and subarachnoidal hemorrhage[23,48,59,60] but not exclusively, since microglial expression of HO-1 was also reported following focal brain ischemia[57,58]. Thus, HO-1 activity can be induced in microglia to breakdown hemoproteins contained in foreign engulfed material during recovery of brain ischemia.

In stroke patients, HO-1 immunoreactivity was mainly observed in astrocytes, occurring within the first 24 h post-infarction, increasing to moderate intensity after few weeks and returning to baseline levels after several months. HO-1 expression in microglia was weaker after focal

Model	*In vitro* cultures			*In vivo* models	
Cell type	Astrocytes	Neurons	Vascular endothelial cells	Transgenic mice	Brain injury in the rat
HO-1 induction by exogenous stimuli	Contradictory Toxic (iron) Protective (Hb, H_2O_2)	Toxic (Hb)	Protective (glucose overload and oxidative stress)		Focal ischemia: expressed in neurons, astrocytes and microglia, TBI: expressed in astrocytes and microglia
HO-1 knock-out				Massive iron deposition in the liver and death	
HO-2 knock-in		Protective			
HO-2 knock-out	Protective (Hb)	Protective (hemin)		Increased neuronal death (upon MCA-O and TBI)	
Bilirubin (physiologic [ ])			Protective, anti-inflammatory		Protective (EAE)
Bilirubin (UB) (increased [ ])	Toxic (also for microglia)	Toxic			
BVR					Expressed in neurons (MCA-O)
BOXes			Toxic		Vasospasm

Table 2. Contradictory results of experimental studies employing markers of heme degradation. Legend: HO – heme oxygenases, Hb – hemoglobin, H_2O_2 – hydrogen peroxide, TBI – traumatic brain injury, MCA-O – middle cerebral artery occlusion, UB – unconjugated bilirubin, [ ] – concentrations, EAE – experimental autoimmune encephalitis, BVR – biliverdin reductase, BOXes – bilirubin oxidation products. In parenteses ( ), stimuli applied to cell culture or experimental animal model.

brain ischemia and also appeared in the first 24 h. Weak-to-moderate HO-1 staining was still present in some neurons months after the infarction. The results of this clinical study also pointed that the presence of hemorrhage as a complication of the initial injury was predictive of HO-1 expression in microglia[111].

In traumatic brain injury patients, HO-1 expression was mainly located in activated microglia in the lesion borders by 6 h, increased within the first 24 h and remained detectable until 6 months post-TBI[111]. Rare, faintly stained HO-1$^+$ astrocytes were also observed until 16 days post-TBI, disappearing over the course of 3 weeks. Weak-to-moderate HO-1 expression was also found in neurons around the lesion between 6 and 24 h following TBI.

In patients recovering from subarachnoidal hemorrhage, oxidation products of bilirubin and biliverdin (BOXes) were found to increase in the cerebrospinal fluid, peaking at about 6 to 8 days, and to be associated with delayed vasospasm [112]. It has been thus hypothesized that BOXes produced in blood clots are capable of inducing constriction of vascular smooth muscle cells and to damage the contractile elements of these cells[109]. However,

carbon monoxide resulting from heme oxygenase activity was found to decrease vasospasm following TBI[12,48,113]. The antioxidant effect of bilirubin at low concentrations may also counteract vasospasm by decreasing oxidative stress, which suggests a regulation of vasospasm by BOX and bilirubin balance[109].

Finally, increased brain iron sequestration and oxidative mitochondrial injury were reported in a variety of neurological diseases caused by neurodegeneration (Alzheimer's disease, Parkinson's disease, progressive supranuclear palsy), metabolic disturbances (PANK-2 deficiency, aceruloplasminemia) and immunologic/infectious diseases (multiple sclerosis, HIV-1 encephalitis) [11, 114]. This is suggestive of prolonged HO-1 activation and iron production leading to enhanced neurodegeneration in these pathologies[114]. Interestingly, research in Alzheimer's disease demonstrated a number of single-point mutations in amyloid precursor proteins (APP) binding HO-1 and HO-2 that decrease overall HO activity, lower bilirubin availability and increase neurotoxicity[115].

5. THERAPEUTIC IMPLICATIONS

At present, the modulation of HO-1 activity and iron chelation are the main therapeutical approaches under investigation. HO-1 was found to be modulated by metalloporphyrin inhibitors of HO activity[116], but so far, results have contradicted the neuroprotective role of HO-1. For instance, the administration of HO inhibitors like tin protoporphyrin were able to decrease damage to the hippocampus caused by ischemia, hemorrhage or trauma and to reduce brain edema[117-119]. This is in contrast with the observed reduction in SOD-2 expression caused by tin protoporphyrin[76] (see *section 2.5*), which would potentially decrease antioxidant scavenging and render the hippocampus vulnerable to oxidative stress.

Following subarachnoidal hemorrhage, one can speculate that HO-1 inhibition may decrease the formation of bilirubin and decrease the formation of vasospasm-inducing BOXes. Iron chelators like deferoxamine were also capable of decreasing edema following intracranial bleeding[117,120]. Another iron chelator, DP-b99, is currently under study in a stroke multicenter clinical trial[121].

Another therapeutic possibility relies on the modulation of the NO synthases, which could yield additional effects on HO-1 activity. In this regard, s-nitroso-n-acetylpenicillamine, a known activator of NO synthase, was found to increase HO-1 activity[122]. Cobalt protoporphyrin (CoPP) was also shown to stimulate HO-1 activity, to decrease iNOS and increase eNOS expressions, improving vascular relaxation in the presence of diabetic vasculopathy[123].

Thus, even though it is still under investigation whether HO-1 activity and its products are beneficial or detrimental during recovery of focal brain ischemia, the pathways of heme degradation may constitute an interesting therapeutical target.

6. Conclusions

The novel findings on heme degradation mechanisms and its implications on the recovery of acute brain

Figura 9

injury are dependent on the initial insult to the brain. In general, HO-1 induction seems to be beneficial in situations of ischemia and trauma, but not in situations of hemorrhage to the brain parenchyma, since it elicits a more pronounced and protracted inflammatory response in local microglia and promotes the oxidation of heme and bilirubin by free radicals. The benefits of HO-1 activity also rely on the sequestration of heme, which reduces the activity of pro-inflammatory enzymes that require heme for their activity such as NOS and COX. Evidence for a neuroprotective role of HO-1 in acute brain injury seems consistent with experimental findings *in vivo,* whereas some *in vitro* studies indicate otherwise. Therapeutical investigations show a beneficial effect of HO-1 inhibition and iron chelation in the reduction of edema and delayed neurodegeneration induced by brain injury. These contradictory findings may reside on the duration and localization of HO-1 upregulation. The pro-oxidant effect of HO-1 has been attributed to iron release and to the inability of neurons, but not of astrocytes, to sequester and detoxify excess iron. On the long run, persistent HO-1 activity may lead to progressive iron deposition in neurons and astroglia, as reported in a variety of chronic neurodegenerative disorders, and to oxidative stress in mitochondria. Thus, the development of therapies directed to heme degradation must take into account the beneficial effects of HO-1 and the detrimental, pro-oxidant actions of free radicals and free iron in the acute phase of brain injury. Results from clinical trials with iron chelators, among others, are thus awaited with renewed interest.

ACKNOWLEDGEMENTS

Based on the doctoral thesis "*Recovery from Focal Brain Ischemia Induced by Extradural Compression in Diabetic and Non-diabetic Rats*" by the same author (ISBN: 91-7140-722-7; available online at http://diss.kib.ki.se/2006/91-7140-722-7). T.P.M. was supported by FCT- Fundação para a Ciência e Tecnologia (European Social Fund QCA III, 2000-2006) and Fundação Gulbenkian (Portugal), and funds from the Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden.

REFERENCES

1. Chang EF, Claus CP, Vreman HJ, Wong RJ, Noble-Haeusslein LJ. Heme regulation in traumatic brain injury: Relevance to the adult and developing brain. *J Cereb Blood Flow Metab*. 2005;25:1401-1417
2. Hua Y, Xi G, Keep RF, Hoff JT. Complement activation in the brain after experimental intracerebral hemorrhage. *J Neurosurg*. 2000;92:1016--1022
3. Macdonald RL, Weir BK. A review of hemoglobin and the pathogenesis of cerebral vasospasm. *Stroke*. 1991;22:971-982
4. Wagner KR, Sharp FR, Ardizzone TD, Lu A, Clark JF. Heme and iron metabolism: Role in cerebral hemorrhage. *J Cereb Blood Flow Metab*. 2003;23:629-652
5. Xi G, Keep RF, Hoff JT. Erythrocytes and delayed brain edema formation following intracerebral hemorrhage in rats. *J Neurosurg*. 1998;89:991-996
6. Sharp FR, Massa SM, Swanson RA. Heat-shock protein protection. *Trends Neurosci*. 1999;22:97-99
7. Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc Natl Acad Sci U S A*. 1968;61:748-755
8. Tenhunen R, Marver HS, Schmid R. Microsomal heme oxygenase. Characterization of the enzyme. *J Biol Chem*. 1969;244:6388-6394
9. Maines MD, Kappas A. Cobalt induction of hepatic heme oxygenase; with evidence that cytochrome p-450 is not essential for this enzyme activity. *Proc Natl Acad Sci U S A*. 1974;71:4293-4297
10. Kranc KR, Pyne GJ, Tao L, Claridge TD, Harris DA, Cadoux-Hudson TA, Turnbull JJ, Schofield CJ, Clark JF. Oxidative degradation of bilirubin produces vasoactive compounds. *Eur J Biochem*. 2000;267:7094-7101

11. Maines MD. The heme oxygenase system and its functions in the brain. *Cell Mol Biol (Noisy-le-grand)*. 2000;46:573-585
12. Tanaka T, Nishimura Y, Tsunoda H, Naka M. Pharmacogenomics and therapeutic target validation in cerebral vasospasm. *J Cardiovasc Pharmacol*. 2000;36 Suppl 2:S1-4
13. Maines MD. The heme oxygenase system: A regulator of second messenger gases. *Annu Rev Pharmacol Toxicol*. 1997;37:517-554
14. Rouault TA. Systemic iron metabolism: A review and implications for brain iron metabolism. *Pediatr Neurol*. 2001;25:130-137
15. Regan RF, Kumar N, Gao F, Guo Y. Ferritin induction protects cortical astrocytes from heme-mediated oxidative injury. *Neuroscience*. 2002;113:985-994
16. Dennery PA, Visner G, Weng YH, Nguyen X, Lu F, Zander D, Yang G. Resistance to hyperoxia with heme oxygenase-1 disruption: Role of iron. *Free Radic Biol Med*. 2003;34:124-133
17. Shibahara S, Muller R, Taguchi H, Yoshida T. Cloning and expression of cdna for rat heme oxygenase. *Proc Natl Acad Sci U S A*. 1985;82:7865-7869
18. Rotenberg MO, Maines MD. Isolation, characterization, and expression in escherichia coli of a cdna encoding rat heme oxygenase-2. *J Biol Chem*. 1990;265:7501-7506
19. Ewing JF, Maines MD. Glutathione depletion induces heme oxygenase-1 (hsp32) mrna and protein in rat brain. *J Neurochem*. 1993;60:1512-1519
20. Ferris CD, Jaffrey SR, Sawa A, Takahashi M, Brady SD, Barrow RK, Tysoe SA, Wolosker H, Baranano DE, Dore S, Poss KD, Snyder SH. Haem oxygenase-1 prevents cell death by regulating cellular iron. *Nat Cell Biol*. 1999;1:152-157
21. Fukuda K, Richmon JD, Sato M, Sharp FR, Panter SS, Noble LJ. Induction of heme oxygenase-1 (ho-1) in glia after traumatic brain injury. *Brain Res*. 1996;736:68-75
22. Geddes JW, Pettigrew LC, Holtz ML, Craddock SD, Maines MD. Permanent focal and transient global cerebral ischemia increase glial and neuronal expression of heme oxygenase-1, but not heme oxygenase-2, protein in rat brain. *Neurosci Lett*. 1996;210:205-208
23. Turner CP, Bergeron M, Matz P, Zegna A, Noble LJ, Panter SS, Sharp FR. Heme oxygenase-1 is induced in glia throughout brain by subarachnoid hemoglobin. *J Cereb Blood Flow Metab*. 1998;18:257-273
24. McCoubrey WK, Jr., Huang TJ, Maines MD. Isolation and characterization of a cdna from the rat brain that encodes hemoprotein heme oxygenase-3. *Eur J Biochem*. 1997;247:725-732
25. Hayashi S, Omata Y, Sakamoto H, Higashimoto Y, Hara T, Sagara Y, Noguchi M. Characterization of rat heme oxygenase-3 gene. Implication of processed pseudogenes derived from heme oxygenase-2 gene. *Gene*. 2004;336:241-250
26. Scapagnini G, D'Agata V, Calabrese V, Pascale A, Colombrita C, Alkon D, Cavallaro S. Gene expression profiles of heme oxygenase isoforms in the rat brain. *Brain Res*. 2002;954:51-59
27. Dennery PA. Regulation and role of heme oxygenase in oxidative injury. *Curr Top Cell Regul*. 2000;36:181-199
28. Schipper HM. Heme oxygenase-1: Role in brain aging and neurodegeneration. *Exp Gerontol*. 2000;35:821-830
29. Matsuoka Y, Kitamura Y, Okazaki M, Sakata M, Tsukahara T, Taniguchi T. Induction of heme oxygenase-1 and major histocompatibility complex antigens in transient forebrain ischemia. *J Cereb Blood Flow Metab*. 1998;18:824-832
30. Lavrovsky Y, Schwartzman ML, Levere RD, Kappas A, Abraham NG. Identification of binding sites for transcription factors nf-kappa b and ap-2 in the promoter region of the human heme oxygenase 1 gene. *Proc Natl Acad Sci U S A*. 1994;91:5987-5991
31. Muller RM, Taguchi H, Shibahara S. Nucleotide sequence and organization of the rat heme oxygenase gene. *J Biol Chem*. 1987;262:6795-6802
32. Weber CM, Eke BC, Maines MD. Corticosterone regulates heme oxygenase-2 and no synthase transcription and protein expression in rat brain. *J Neurochem*. 1994;63:953-962
33. Maines MD, Eke BC, Zhao X. Corticosterone promotes increased heme oxygenase-2 protein and transcript expression in the newborn rat brain. *Brain Res*. 1996;722:83-94
34. McCoubrey WK, Jr., Maines MD. The structure, organization and differential expression of the gene encoding rat heme oxygenase-2. *Gene*. 1994;139:155-161
35. Raju VS, McCoubrey WK, Jr., Maines MD. Regulation of heme oxygenase-2 by glucocorticoids in neonatal rat brain: Characterization of a functional glucocorticoid response element. *Biochim Biophys Acta*. 1997;1351:89-104
36. Dore S, Takahashi M, Ferris CD, Zakhary R, Hester LD, Guastella D, Snyder SH. Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury. *Proc Natl Acad Sci U S A*. 1999;96:2445-2450
37. Trakshel GM, Maines MD. Multiplicity of heme oxygenase isozymes. Ho-1 and ho-2 are different molecular species in rat and rabbit. *J Biol Chem*. 1989;264:1323-1328
38. Sun Y, Rotenberg MO, Maines MD. Developmental expression of heme oxygenase isozymes in rat brain. Two ho-2 mrnas are detected. *J Biol Chem*. 1990;265:8212-8217
39. Chang EF, Wong RJ, Vreman HJ, Igarashi T, Galo E, Sharp FR, Stevenson DK, Noble-Haeusslein LJ. Heme oxygenase-2 protects against lipid peroxidation-mediated cell loss and impaired motor recovery after traumatic brain injury. *J Neurosci*. 2003;23:3689-3696
40. Ewing JF, Haber SN, Maines MD. Normal and heat-induced patterns of expression of heme oxygenase-1 (hsp32) in rat brain: Hyperthermia causes rapid induction of mrna and protein. *J Neurochem*. 1992;58:1140-1149
41. Vincent SR, Das S, Maines MD. Brain heme oxygenase isoenzymes and nitric oxide synthase are co-localized in select neurons. *Neuroscience*. 1994;63:223-231
42. Ewing JF, Maines MD. Histochemical localization of heme oxygenase-2 protein and mrna expression in rat brain. *Brain Res Brain Res Protoc*. 1997;1:165-174
43. Applegate LA, Luscher P, Tyrrell RM. Induction of heme oxygenase: A general response to oxidant stress in cultured mammalian cells. *Cancer Res*. 1991;51:974-978

44. Dwyer BE, Nishimura RN, Lu SY. Differential expression of heme oxygenase-1 in cultured cortical neurons and astrocytes determined by the aid of a new heme oxygenase antibody. Response to oxidative stress. *Brain Res Mol Brain Res*. 1995;30:37-47
45. Ewing JF, Maines MD. Rapid induction of heme oxygenase 1 mrna and protein by hyperthermia in rat brain: Heme oxygenase 2 is not a heat shock protein. *Proc Natl Acad Sci U S A*. 1991;88:5364-5368
46. Smith MA, Kutty RK, Richey PL, Yan SD, Stern D, Chader GJ, Wiggert B, Petersen RB, Perry G. Heme oxygenase-1 is associated with the neurofibrillary pathology of alzheimer's disease. *Am J Pathol*. 1994;145:42-47
47. Koistinaho J, Miettinen S, Keinanen R, Vartiainen N, Roivainen R, Laitinen JT. Long-term induction of haem oxygenase-1 (hsp-32) in astrocytes and microglia following transient focal brain ischaemia in the rat. *Eur J Neurosci*. 1996;8:2265-2272
48. Matz P, Turner C, Weinstein PR, Massa SM, Panter SS, Sharp FR. Heme-oxygenase-1 induction in glia throughout rat brain following experimental subarachnoid hemorrhage. *Brain Res*. 1996;713:211-222
49. Galbraith R. Heme oxygenase: Who needs it? *Proc Soc Exp Biol Med*. 1999;222:299-305
50. Suttner DM, Dennery PA. Reversal of ho-1 related cytoprotection with increased expression is due to reactive iron. *Faseb J*. 1999;13:1800--1809
51. Bhasin RR, Xi G, Hua Y, Keep RF, Hoff JT. Experimental intracerebral hemorrhage: Effect of lysed erythrocytes on brain edema and blood-brain barrier permeability. *Acta Neurochir Suppl*. 2002;81:249-251
52. Hoff JT, Xi G. Brain edema from intracerebral hemorrhage. *Acta Neurochir Suppl*. 2003;86:11--15
53. Wu J, Hua Y, Keep RF, Nakamura T, Hoff JT, Xi G. Iron and iron-handling proteins in the brain after intracerebral hemorrhage. *Stroke*. 2003;34:2964-2969
54. Xi G, Hua Y, Bhasin RR, Ennis SR, Keep RF, Hoff JT. Mechanisms of edema formation after intracerebral hemorrhage: Effects of extravasated red blood cells on blood flow and blood-brain barrier integrity. *Stroke*. 2001;32:2932--2938
55. Kaneko Y, Kitamoto T, Tateishi J, Yamaguchi K. Ferritin immunohistochemistry as a marker for microglia. *Acta Neuropathol (Berl)*. 1989;79:129-136
56. Takeda A, Onodera H, Sugimoto A, Itoyama Y, Kogure K, Shibahara S. Increased expression of heme oxygenase mrna in rat brain following transient forebrain ischemia. *Brain Res*. 1994;666:120-124
57. Koistinaho J, Miettinen S, Keinanen R, Vartiainen N, Roivainen R, Laitinen JT. Long-term induction of heme oxygenase-1 (hsp-32) in astrocytes and microglia following transient focal brain ischaemia in the rat. *Eur J Neurosci*. 1996;8:2265-2272
58. Bergeron M, Ferriero DM, Vreman HJ, Stevenson DK, Sharp FR. Hypoxia-ischemia, but not hypoxia alone, induces the expression of heme oxygenase-1 (hsp32) in newborn rat brain. *J Cereb Blood Flow Metab*. 1997;17:647-658
59. Mautes AE, Kim DH, Sharp FR, Panter S, Sato M, Maida N, Bergeron M, Guenther K, Noble LJ. Induction of heme oxygenase-1 (ho-1) in the contused spinal cord of the rat. *Brain Res*. 1998;795:17-24
60. Yi JH, Hazell AS. N-acetylcysteine attenuates early induction of heme oxygenase-1 following traumatic brain injury. *Brain Res*. 2005;1033:13-19
61. Dwyer BE, Nishimura RN, Lu SY, Alcaraz A. Transient induction of heme oxygenase after cortical stab wound injury. *Brain Res Mol Brain Res*. 1996;38:251-259
62. Song W, Su H, Song S, Paudel HK, Schipper HM. Over-expression of heme oxygenase-1 promotes oxidative mitochondrial damage in rat astroglia. *J Cell Physiol*. 2006;206:655-663
63. Regan RF, Guo Y, Kumar N. Heme oxygenase-1 induction protects murine cortical astrocytes from hemoglobin toxicity. *Neurosci Lett*. 2000;282:1-4
64. Sacerdoti D, Olszanecki R, Li Volti G, Colombrita C, Scapagnini G, Abraham NG. Heme oxygenase overexpression attenuates glucose-mediated oxidative stress in quiescent cell phase: Linking heme to hyperglycemia complications. *Curr Neurovasc Res*. 2005;2:103-111
65. Chen-Roetling J, Regan RF. Effect of heme oxygenase-1 on the vulnerability of astrocytes and neurons to hemoglobin. *Biochem Biophys Res Commun*. 2006;350:233-237
66. Regan RF, Chen J, Benvenisti-Zarom L. Heme oxygenase-2 gene deletion attenuates oxidative stress in neurons exposed to extracellular hemin. *BMC Neurosci*. 2004;5:34
67. Rogers B, Yakopson V, Teng ZP, Guo Y, Regan RF. Heme oxygenase-2 knockout neurons are less vulnerable to hemoglobin toxicity. *Free Radic Biol Med*. 2003;35:872-881
68. Poss KD, Tonegawa S. Heme oxygenase 1 is required for mammalian iron reutilization. *Proc Natl Acad Sci U S A*. 1997;94:10919-10924
69. Dore S, Sampei K, Goto S, Alkayed NJ, Guastella D, Blackshaw S, Gallagher M, Traystman RJ, Hurn PD, Koehler RC, Snyder SH. Heme oxygenase-2 is neuroprotective in cerebral ischemia. *Mol Med*. 1999;5:656-663
70. Dore S, Goto S, Sampei K, Blackshaw S, Hester LD, Ingi T, Sawa A, Traystman RJ, Koehler RC, Snyder SH. Heme oxygenase-2 acts to prevent neuronal death in brain cultures and following transient cerebral ischemia. *Neuroscience*. 2000;99:587-592
71. Dore S, Snyder SH. Neuroprotective action of bilirubin against oxidative stress in primary hippocampal cultures. *Ann N Y Acad Sci*. 1999;890:167-172
72. Maines MD, Polevoda B, Coban T, Johnson K, Stoliar S, Huang TJ, Panahian N, Cory-Slechta DA, McCoubrey WK, Jr. Neuronal overexpression of heme oxygenase-1 correlates with an attenuated exploratory behavior and causes an increase in neuronal nadph diaphorase staining. *J Neurochem*. 1998;70:2057-2069
73. Turkseven S, Kruger A, Mingone CJ, Kaminski P, Inaba M, Rodella LF, Ikehara S, Wolin MS, Abraham NG. Antioxidant mechanism of heme

oxygenase-1 involves an increase in superoxide dismutase and catalase in experimental diabetes. *Am J Physiol Heart Circ Physiol*. 2005;289:H701-707
74. Otterbein LE, Choi AM. Heme oxygenase: Colors of defense against cellular stress. *Am J Physiol Lung Cell Mol Physiol*. 2000;279:L1029-1037
75. Keller JN, Kindy MS, Holtsberg FW, St Clair DK, Yen HC, Germeyer A, Steiner SM, Bruce-Keller AJ, Hutchins JB, Mattson MP. Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: Suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. *J Neurosci*. 1998;18:687-697
76. Son E, Jeong J, Lee J, Jung DY, Cho GJ, Choi WS, Lee MS, Kim SH, Kim IK, Suk K. Sequential induction of heme oxygenase-1 and manganese superoxide dismutase protects cultured astrocytes against nitric oxide. *Biochem Pharmacol*. 2005;70:590-597
77. Frankel D, Mehindate K, Schipper HM. Role of heme oxygenase-1 in the regulation of manganese superoxide dismutase gene expression in oxidatively-challenged astroglia. *J Cell Physiol*. 2000;185:80-86
78. Tsubokura S, Watanabe Y, Ehara H, Imamura K, Sugimoto O, Kagamiyama H, Yamamoto S, Hayaishi O. Localization of prostaglandin endoperoxide synthase in neurons and glia in monkey brain. *Brain Res*. 1991;543:15-24
79. Haider A, Olszanecki R, Gryglewski R, Schwartzman ML, Lianos E, Kappas A, Nasjletti A, Abraham NG. Regulation of cyclooxygenase by the heme-heme oxygenase system in microvessel endothelial cells. *J Pharmacol Exp Ther*. 2002;300:188-194
80. Pucci ML, Chakkalakkal B, Liclican EL, Leedom AJ, Schuster VL, Abraham NG. Augmented heme oxygenase-1 induces prostaglandin uptake via the prostaglandin transporter in micro-vascular endothelial cells. *Biochem Biophys Res Commun*. 2004;323:1299-1305
81. Huang TJ, Trakshel GM, Maines MD. Detection of 10 variants of biliverdin reductase in rat liver by two-dimensional gel electrophoresis. *J Biol Chem*. 1989;264:7844-7849
82. Maines MD, Polevoda BV, Huang TJ, McCoubrey WK, Jr. Human biliverdin ixalpha reductase is a zinc-metalloprotein. Characterization of purified and escherichia coli expressed enzymes. *Eur J Biochem*. 1996;235:372-381
83. Maines MD, Trakshel GM. Purification and characterization of human biliverdin reductase. *Arch Biochem Biophys*. 1993;300:320-326
84. Yamaguchi T, Komoda Y, Nakajima H. Biliverdin-ix alpha reductase and biliverdin-ix beta reductase from human liver. Purification and characterization. *J Biol Chem*. 1994;269:24343--24348
85. Kutty RK, Maines MD. Purification and characterization of biliverdin reductase from rat liver. *J Biol Chem*. 1981;256:3956-3962
86. Ewing JF, Weber CM, Maines MD. Biliverdin reductase is heat resistant and coexpressed with constitutive and heat shock forms of heme oxygenase in brain. *J Neurochem*. 1993;61:1015--1023
87. Singleton JW, Laster L. Biliverdin reductase of guinea pig liver. *J Biol Chem*. 1965;240:4780-4789
88. Baranano DE, Snyder SH. Neural roles for heme oxygenase: Contrasts to nitric oxide synthase. *Proc Natl Acad Sci U S A*. 2001;98:10996-11002
89. Hsia DY, Allen FH, Jr., Gellis SS, Diamond LK. Erythroblastosis fetalis. Viii. Studies of serum bilirubin in relation to kernicterus. *N Engl J Med*. 1952;247:668-671
90. Fernandes A, Silva RF, Falcao AS, Brito MA, Brites D. Cytokine production, glutamate release and cell death in rat cultured astrocytes treated with unconjugated bilirubin and lps. *J Neuroimmunol*. 2004;153:64-75
91. Fernandes A, Falcao AS, Silva RF, Gordo AC, Gama MJ, Brito MA, Brites D. Inflammatory signalling pathways involved in astroglial activation by unconjugated bilirubin. *J Neurochem*. 2006;96:1667-1679
92. Gordo AC, Falcao AS, Fernandes A, Brito MA, Silva RF, Brites D. Unconjugated bilirubin activates and damages microglia. *J Neurosci Res*. 2006;84:194-201
93. Stocker R, Glazer AN, Ames BN. Antioxidant activity of albumin-bound bilirubin. *Proc Natl Acad Sci U S A*. 1987;84:5918-5922
94. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science*. 1987;235:1043-1046
95. Haga Y, Tempero MA, Kay D, Zetterman RK. Intracellular accumulation of unconjugated bilirubin inhibits phytohemagglutin-induced proliferation and interleukin-2 production of human lymphocytes. *Dig Dis Sci*. 1996;41:1468-1474
96. McDonagh AF. Is bilirubin good for you? *Clin Perinatol*. 1990;17:359-369
97. Nakagami T, Taji S, Takahashi M, Yamanishi K. Antiviral activity of a bile pigment, biliverdin, against human herpesvirus 6 (hhv-6) in vitro. *Microbiol Immunol*. 1992;36:381-390
98. Nakagami T, Toyomura K, Kinoshita T, Morisawa S. A beneficial role of bile pigments as an endogenous tissue protector: Anti-complement effects of biliverdin and conjugated bilirubin. *Biochim Biophys Acta*. 1993;1158:189-193
99. Hansen TW, Mathiesen SB, Walaas SI. Bilirubin has widespread inhibitory effects on protein phosphorylation. *Pediatr Res*. 1996;39:1072-1077
100. Kwak JY, Takeshige K, Cheung BS, Minakami S. Bilirubin inhibits the activation of superoxide-producing nadph oxidase in a neutrophil cell-free system. *Biochim Biophys Acta*. 1991;1076:369-373
101. Kawamura K, Ishikawa K, Wada Y, Kimura S, Matsumoto H, Kohro T, Itabe H, Kodama T, Maruyama Y. Bilirubin from heme oxygenase-1 attenuates vascular endothelial activation and dysfunction. *Arterioscler Thromb Vasc Biol*. 2005;25:155-160
102. Hayashi S, Takamiya R, Yamaguchi T, Matsumoto K, Tojo SJ, Tamatani T, Kitajima M, Makino N, Ishimura Y, Suematsu M. Induction of heme oxygenase-1 suppresses venular leukocyte adhesion elicited by oxidative stress: Role of bilirubin generated by the enzyme. *Circ Res*. 1999;85:663-671

103. Ishikawa K, Sugawara D, Wang X, Suzuki K, Itabe H, Maruyama Y, Lusis AJ. Heme oxygenase-1 inhibits atherosclerotic lesion formation in ldl-receptor knockout mice. *Circ Res*. 2001;88:506-512
104. Panahian N, Yoshiura M, Maines MD. Overexpression of heme oxygenase-1 is neuroprotective in a model of permanent middle cerebral artery occlusion in transgenic mice. *J Neurochem*. 1999;72:1187-1203
105. Panahian N, Huang T, Maines MD. Enhanced neuronal expression of the oxidoreductase—biliverdin reductase—after permanent focal cerebral ischemia. *Brain Res*. 1999;850:1-13
106. Panahian N, Maines MD. Assessment of induction of biliverdin reductase in a mouse model of middle cerebral artery occlusion. *Brain Res Brain Res Protoc*. 2000;6:53-70
107. Liu Y, Zhu B, Wang X, Luo L, Li P, Paty DW, Cynader MS. Bilirubin as a potent antioxidant suppresses experimental autoimmune encephalomyelitis: Implications for the role of oxidative stress in the development of multiple sclerosis. *J Neuroimmunol*. 2003;139:27-35
108. Wang WW, Smith DL, Zucker SD. Bilirubin inhibits inos expression and no production in response to endotoxin in rats. *Hepatology*. 2004;40:424-433
109. Clark JF, Sharp FR. Bilirubin oxidation products (boxes) and their role in cerebral vasospasm after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2006;26:1223-1233
110. Clark JF, Reilly M, Sharp FR. Oxidation of bilirubin produces compounds that cause prolonged vasospasm of rat cerebral vessels: A contributor to subarachnoid hemorrhage-induced vasospasm. *J Cereb Blood Flow Metab*. 2002;22:472-478
111. Beschorner R, Adjodah D, Schwab JM, Mittelbronn M, Pedal I, Mattern R, Schluesener HJ, Meyermann R. Long-term expression of heme oxygenase-1 (ho-1, hsp-32) following focal cerebral infarctions and traumatic brain injury in humans. *Acta Neuropathol (Berl)*. 2000;100:377-384
112. Pyne-Geithman GJ, Morgan CJ, Wagner K, Dulaney EM, Carrozzella J, Kanter DS, Zuccarello M, Clark JF. Bilirubin production and oxidation in csf of patients with cerebral vasospasm after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2005;25:1070-1077
113. Suzuki H, Kanamaru K, Tsunoda H, Inada H, Kuroki M, Sun H, Waga S, Tanaka T. Heme oxygenase-1 gene induction as an intrinsic regulation against delayed cerebral vasospasm in rats. *J Clin Invest*. 1999;104:59-66
114. Schipper HM. Heme oxygenase expression in human central nervous system disorders. *Free Radic Biol Med*. 2004;37:1995-2011
115. Takahashi M, Dore S, Ferris CD, Tomita T, Sawa A, Wolosker H, Borchelt DR, Iwatsubo T, Kim SH, Thinakaran G, Sisodia SS, Snyder SH. Amyloid precursor proteins inhibit heme oxygenase activity and augment neurotoxicity in alzheimer's disease. *Neuron*. 2000;28:461-473
116. Maines MD. Zinc . Protoporphyrin is a selective inhibitor of heme oxygenase activity in the neonatal rat. *Biochim Biophys Acta*. 1981;673:339-350
117. Huang FP, Xi G, Keep RF, Hua Y, Nemoianu A, Hoff JT. Brain edema after experimental intracerebral hemorrhage: Role of hemoglobin degradation products. *J Neurosurg*. 2002;96:287-293
118. Kadoya C, Domino EF, Yang GY, Stern JD, Betz AL. Preischemic but not postischemic zinc protoporphyrin treatment reduces infarct size and edema accumulation after temporary focal cerebral ischemia in rats. *Stroke*. 1995;26:1035--1038
119. Panizzon KL, Dwyer BE, Nishimura RN, Wallis RA. Neuroprotection against ca1 injury with metalloporphyrins. *Neuroreport*. 1996;7:662--666
120. Nakamura T, Keep RF, Hua Y, Schallert T, Hoff JT, Xi G. Deferoxamine-induced attenuation of brain edema and neurological deficits in a rat model of intracerebral hemorrhage. *J Neurosurg*. 2004;100:672-678
121. Ferro JM, Davalos A. Other neuroprotective therapies on trial in acute stroke. *Cerebrovasc Dis*. 2006;21 Suppl 2:127-130
122. Motterlini R, Foresti R, Intaglietta M, Winslow RM. No-mediated activation of heme oxygenase: Endogenous cytoprotection against oxidative stress to endothelium. *Am J Physiol*. 1996;270:H107-114
123. Di Pascoli M, Rodella L, Sacerdoti D, Bolognesi M, Turkseven S, Abraham NG. Chronic co levels has a beneficial effect on vascular relaxation in diabetes. *Biochem Biophys Res Commun*. 2006;340:935-943