The Janus face of the heme oxygenase/biliverdin reductase system in Alzheimer disease: It's time for reconciliation

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Eugenio Barone [a,1,2], Fabio Di Domenico [b,2], Cesare Mancuso [c], D. Allan Butterfield [a,]

[a] Department of Chemistry, Center of Membrane Sciences, Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40506-0055, USA

[b] Department of Biochemical Scivl(2z.2)igiB4foSaemduces the intracellular levels of the free radical scavengers
biliverdin-IX alpha (BV)/bilirubin-IX alpha (BR) as well as the pleiotropic gaseous neuromodulator carbon monoxide (CO) and ferrous iron. Two main and opposite hypotheses for a role of the HO-1/BVR-A system in AD propose that this system mediates neurotoxic and neuroprotective

function (Hayashi et al., 2004) (McCoubrey et al., 1997). Nevertheless,

and consequent degradation of Keap1, which under normal conditions sequesters Nrf-2 into the cytoplasm, avoiding its transcriptional activity (He et al., 2007; Zenke-Kawasaki et al., 2007) (Fig. 1). Substances and drugs that differently affect HO-1 expression and activity have been extensively reviewed (Mancuso and Barone, 2009). As regard to HO-2 gene regulation, only limited evidence is available to implicate the glucocorticoid responsive elements (GRE) in the gene encoding for HO-2 as the main site involved in the modulation of HO-2 protein levels (see above) (Liu et al., 2000).

In the central nervous system (CNS), HO-2 is expressed in neuronal populations in almost all brain areas (Maines, 1997), whereas HO-1 is present at low levels in sparse groups of neurons, including the ventromedial and paraventricular nuclei of the hypothalamus (Maines, 1997). Heme oxygenase-1 is also found in cells of glial lineage, where its expression can be induced by oxidative stress (Dwyer et al., 1995).

HO-1 and HO-2 catalyze the same reaction, namely the transformation of iron-protoporphyrin-IX-alpha (heme) into equimolar amount of ferrous iron [Fe(II)], carbon monoxide (CO), and biliverdin-IX-alpha (BV-alpha) (Maines, 1997, 2000) (Fig. 1). The activity of both enzymes can be regulated by post-translational modifications such as phosphorylation of specific serine or tyrosine residues (Fig. 2). In particular, HO-1 activity might be regulated through Akt-mediated phosphorylation of Ser188 (Salinas et al., 2004). This kind of phosphorylation may change the strength of binding/interaction between HO-1 and BVR. However, considering the large number of residues involved in the interaction, a large change in binding affinity is not expected for a single phosphory-

delivered to cell, and the tissue-specific signaling transduction pathway(s) involved in its biological activity. Carbon monoxide produced in rat hypothalamus by HO activity has displayed anti-inflammatory activity consisting in the attenuation of KCl-induced interleukin-1β release from interleukinergic neurons (Mancuso et al., 1998). However, hypothalamic CO also reduces stimulated increases in the in vitro and in vivo release of corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) (Mancuso et al., 1997, 1999, 2010; Pozzoli et al., 1994), effects that are clearly pro-inflammatory. Indeed, their end result is decreased pituitary release of adrenocorticotropin hormone (ACTH), which, in turn, stimulates glucocorticoid production and release by the adrenal cortex. Additional evidence of CO's dual role in the CNS has been provided by Luiz Branco's group. In their studies on CO's involvement in the pyrogenic response to stress, intracerebroventricular administration of HO inhibitors decreased LPS-induced fever in rats, while heme overload caused a rise in body temperature (Steiner and Branco, 2000, 2001; Steiner et al., 1999, 2003). In contrast, if the increased CO formation was confined to the locus coeruleus, the febrile response to LPS decreased (Ravanelli et al., 2007). Although it is only indirectly related to inflammation, CO's effect on the release of gonadotropin-releasing-hormone (GnRH) is worth mentioning. Carbon monoxide was shown to up-regulate GnRH release in the hypothalamic GT1-7 cell line, and this effect seems to be dependent on the CO-mediated production of prostaglandin E2 (Errico et al., 2010).

Iron

Iron is a cofactor for several enzymes and is able to modulate specific

of stress-responsive genes such as HO-1 (Tudor et al., 2008) and iNOS (Di Domenico et al., 2013b; Gibbs et al., 2012a) (Figs. 2A and B).

Once activated by the insulin receptor, BVR is able to modulate two of the most important arms of the insulin signaling pathway: MAPK and phosphatidylinositol-3-kinase (PI3K) (Kapitulnik and Maines, 2009). MAPK and PI3K pathways have essential roles in neuronal activity and development: (i) the PI3K pathway is involved in the maintenance of synaptic plasticity and memory consolidation (Horwood et al., 2006), amyloid-β-peptide (Aβ)-induced memory loss (Chiang et al., 2010), synthesis of nitric oxide (NO), which in turn plays a role in learning and memory processes (Calabrese et al., 2007a); and (ii) the MAPK cascade is responsible both for the induction of several genes required for neuronal and synapse growth, maintenance and repair processes, as well as serving as a modulator of hippocampal synaptic plasticity that underlies learning and memory (Akter et al., 2011). Consequently, it is clear that the broad spectrum of pleiotropic actions mediated by BVR-A makes this enzyme an important interventional target for the development of new therapeutic strategies.

As with HO-1, also BVR at the beginning was considered relevant only for its ability to produce BR. However, in light of this pleiotropic nature of BVR, the discrimination between the effects directly mediated by BVR and those mediated by BR become essential in order to better understand and clarify the broad spectrum of actions to which we refer when we mention the HO/BVR system.

Bilirubin is a linear tetrapyrrole, characterized by high lipophylicity, and was extensively studied for its antioxidant and antinitrosative properties (Barone et al., 2009; Dore et al., 1999; Mancuso et al., 2003; Stocker et al., 1987a,b; Takahashi et al., 2000). Despite this important antioxidant behavior, if produced in excess, as during hemolytic anemia or sepsis, unconjugated BR becomes neurotoxic through multiple mechanisms involving the disruption of cell membrane structure, the reduction of mitochondrial transmembrane potential and the activation of the apoptotic cascade (Brito et al., 2004; Kapitulnik, 2004).

Other than its antioxidant activity, BR increased neuronal NOS expression and nitric oxide formation in both primary cultures of cerebellar granule neurons and neurotrophin-sensitive PC12 cells (Mancuso et al., 2008), and it was shown that this gaseous neurotransmitter plays a key role in the long-term potentiation and synaptic plasticity (Calabrese et al., 200-). In addition, in PC12 cells BR upregulated CREB (Mancuso et al., 2008), which is considered an important transcription factor regulating both short- and long- term memory (Suzuki et al., 2011).

Al14(x[(Al14nu]TJ 0 09)(in)-1(e)-3dv [(i)-n53tl9(n)-8p2 0 T -0.4-12(-3(e)71e.)Tj /T1_2)(in)-0 -1..ip(i)6(s)-275(ccgp2 0y:)16aPs e

in the cerebral cortex and cerebral vessels in association with pathological lesions of AD (Premkumar et al., 1995). Later, in 1997, Smith et al. proposed that redox-active iron is associated with the senile plaques and neurofibrillary tangles, indicating that iron accumulation could be an important contributor toward the oxidative damage of Alzheimer disease (Smith et al., 1997), thus providing a basis for the future involvement of HO-1 as one of the main source of iron deposition and accumulation.

In 2000, Schipper et al. found decreased plasma and CSF HO-1 protein and lymphocyte HO-1 mRNA levels in subjects with sporadic AD proposing for the first time, the quantitative assay for lymphocyte HO-1 mRNA expression as a useful biologic marker in early sporadic AD (Schipper et al., 2000) (Table 1). Similarly in 2002, Ishizuka et al. showed plasma HO-1 protein and mononuclear cell HO-1 mRNA levels significantly suppressed in AD subjects compared to controls (Ishizuka et al., 2002) (Table 1). However, the reports of HO-1 plasma levels in AD subjects are controversial as highlighted below in this review (Tables 1 and 2).

The year 2000 was a rich one for findings related to HO-1. First, the ability of APP to bind HO-1 and HO-2 and inhibit their activity was demonstrated (Takahash dA3(m)46 -2ndstgh 3iHOt110the

were found significantly correlated with HO-1 levels in the cortex of MCI and AD subjects (Hascalovici et al., 2009).

The last discovery, which we want to mention in this brief discussion, was reported in 2009, when Kanninen et al. indicated that significant reductions in spatial learning deficits of aged APP/PS1 mice can be achieved by modulating levels of Nrf2 in the brain. This

increased Tyr nitration, a competition between nitration and phosphorylation processes occurs. Certainly, from a chemical point of view, steric hindrance of the NO_2 group on the 3-position of Tyr could significantly modulate activity of Tyr kinases for the 4-OH group. This notion strengthens the hypothesis that nitrosative stress prevents/inhibits Tyr phosphorylation on BVR-A (Barone et al., 2011a,b). The evidence that BVR-A nitration occurred also in the hippocampus of MCI subjects (Table 2), suggests that any modification in terms of cell stress response is an early event in the pathogenesis and progression of AD (Barone et al., 2011b).

These findings raised the question about the effective neuroprotective role of the HO-1/BVR-A system in AD. Why, despite the up-regulation of both HO-1 and BVR-A protein levels, are the oxidative stress marker levels in the hippocampus still higher and the pathological features of AD still present? Based on our results, the answer is quite simple: it is no longer correct to measure only total protein levels as an index to evaluate the involvement of these enzymes in cell stress response since post-translational modifications appear to play a main role in the regulation of the neuroprotective and/or metabolic activities of these proteins. In fact, the observed impairment of BVR-A activity blunts the effects that could be mediated by the up-regulation of this enzyme (Barone et al., 2011a,b).

These observations were logically followed by the analysis of the post-translational modifications of HO-1. The question to be addressed at this stage was to know if only BVR-A was impaired or also HO-1.

We then extended the investigation on the neurobiological features of both HO-1 and HO-2 in the brain of AD and MCI subjects to include: (i) increase of HO-1 protein levels in another well-known brain area involved in AD pathology such as hippocampus; (ii) decrease of HO-2 protein levels in the same brain area; and (iii) the observation that no changes for HO-2 protein levels in cerebellum of MCI subjects were observed (Barone et al., 2012a) (Table 2). Furthermore, we showed a significant increase of Ser-residue phosphorylation along with oxidative post-translational (PC- and HNE-adducts) modifications in the hippocampus of only AD subjects (Barone et al., 2012a) (Table 2). In hippocampus of MCI subjects only a significant increase of HNE-adducts on HO-1 was observed without changes in phosphorylation (Barone et al., 2012a) (Table 2).

Since HO-1 is a stress-inducible protein, the increase of oxidative stress levels in the hippocampus of AD subjects could lead to an increase in HO-1 protein levels and phosphorylation in order to promote its activity and its interaction with BVR (Salinas et al., 2004). At the same time, the increased oxidative stress could be responsible for the observed rise of PC and HNE-adducts, already demonstrated for other proteins in AD (Sultana et al., 2009), including BVR-A (Barone et al., 2011a,b), leading to altered protein structure and function impairment (Butterfield and Lauderback, 2002; Lauderback et al., 2001; Owen et al., 2010; Subramaniam et al., 1997). Based on our experimental model, it is difficult to state which post translational modification precedes the other between phosphorylation and oxidative modification and at least two interpretations could be conceivable: 1) oxidative stress promotes the increase of oxidative damage to HO-1 (e.g., increased PC and HNE-adducts on its structure). Consequently, the cell tries to restore the functionality of the protein by increasing Ser residue phosphorylation; 2) Oxidative stress promotes the increase of Ser-residue phosphorylation in order to activate protein functions, but HO-1 quickly becomes a target for oxidative post-translational modifications, that in turn could impair its function (Barone et al., 2012a) (Fig. 3).

With regard to MCI, the results from hippocampus add new elements to the comprehension of the contribution of the HO-1/BVR-A system ly241̲01ur[(Bu)8(tter)]TJ/Fter

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reflecting improved cognition; and (ii) HO-1 and BVR-A and decreased oxidative/nitrosative stress indices, as well as DLES (Barone et al., 2012b; Butterfield et al., 2012a). Furthermore, BVR-A up-regulation and post-translational modifications significantly correlated with β-secretase protein levels in the brain, suggesting a possible role for BVR-A in Aβ formation (

reductase activity; and (iii) speculatively, activation of both conventional and atypical protein kinase C isoforms (Kapitulnik and Maines, 2009

of HO-1 expression, identified as alpha-1 anti-chymotrypsin (AAT) in patients with sporadic AD. The same inhibition does not occur in CNS of AD patients due to the high AAT exposure to disease-related protein oxidation and nitration. In contrast to the above-referenced studies, a recent research by Mateo et al. (2010) described unaltered HO-1 serum levels between control and AD subjects, and a study (Calabrese et al., 2006) demonstrated increased levels of HO-1 in AD lymphocytes compared with control (Table 2). Extending AD studies from brain to plasma, the Butterfield laboratory investigated the status of the peripheral HO-1/BVR system with the idea that it might reflect brain pathology. We showed, recently, that plasma levels of HO-1 are increased in AD and MCI subjects following disease severity. Our data on plasma HO-1 levels correlate with brain data previously discussed; however, none of the HO-1 aberrant modifications (protein bound-HNE or phosphorylation) seen in brain were found in plasma from AD subjects suggesting that HO-1 analysis may lack AD specificity as a disease biomarker (Di Domenico et al., 2012) (Barone et al., 2012a) (Table 2). The analysis of the literature shows the presence of a number of investigations that propose HO-1 altered expression levels as a biomarker of several different diseases, such as lung function decline in silicosis patients, secondary hemophagocytic syndrome (HPS) or adult-onset Still's disease, type-2 diabetes mellitus and coronary atherosclerosis (Brydun et al., 2007; Miyazaki et al., 2010) (Calabrese et al., 2007b). Thus, altered HO-1 expression in a such number of heterogenic diseases suggests that HO-1 alteration may be specific of an event common to all of these diseases, such as oxidative stress or antioxidant response, but not exclusive of one particular disease.

Mueller et al. (2010) identified the association of BVR-A and -B altered expression in AD and MCI pathology in plasma samples suggesting that the heme degradation pathway, with the focus on BVR, may represent a new avenue for biomarker search (Table 1). Subsequent studies from our laboratory showed that, in AD, data from BVR-A plasma are closely related to BVR-A results in hippocampus with regard to increased protein quantity, increased protein nitration, decreased tyrosine phosphorylation, and decreased protein reductase activity (Di Domenico et al., 2012) (Table 2). Interestingly, we showed that in plasma from probable AD patients (pAD), in which the pro-oxidant conditions are steadily higher than control, BVR-A protein levels and activity follow their relationship seen in hippocampus. Conversely,

In conclusion, the data reported above suggest that even if the blood proteome profile is relatively different from the brain protein profile, the HO-1/BVR-A system status in plasma could mimic the ongoing situation in the brain. Therefore, the analysis of HO-1/BVR-A system in blood-related biofluids might represent a reasonable way to gain information on increased oxidative and nitrosative stress ongoing in the brain. In light of such considerations, the HO-1/BVR-A system could conceivably predict AD onset and advancement, expanding its significance as a potential AD biomarker, from the early stages of the disease, in combination with other AD diagnostic tools.

Future perspective

The role of the HO/BVR axis in the pathogenesis and therapy of AD and MCI is still a developing research area. From a pathogenetic viewpoint, our data confirm a limited neuroprotective role for the HO-1/BVR system in normal brain in terms of enhancement of the cell stress response. However, loss of activity of HO-1/BVR-A leads to loss of neuroprotective BR and the pleiotropic neuroprotective activities of BVR. This loss of function in both enzymes is due to the post-translational modifications on both, under pro-oxidant conditions. On the other

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