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Heme Oxygenase-1 Post-translational Modifications in the Brain of Subjects with Alzheimer Disease and Mild Cognitive Impairment

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Abstract

Alzheimer disease (AD) is a neurodegenerative disorder characterized by progressive cognitive impairment and neuropathology. Oxidative and nitrosative stress play a principal role in the pathogenesis of AD. The induction of the heme oxygenase-1/biliverdin reductase-A (HO-1/BVR-A) system in the brain represents one of the earliest mechanisms activated by cells to counteract the noxious effects of increased reactive oxygen species (ROS) and reactive nitrogen species (RNS). Although initially proposed as a neuroprotective system in AD brain, HO-1/BVR-A pathophysiological features are under debate. We previously reported alterations in BVR activity along with decreased phosphorylation and increased oxidative/nitrosative post-translational modifications in the brain of subjects with AD and mild cognitive impairment (MCI) subjects. Furthermore, other groups proposed the observed increase of HO-1 in AD brain as a possible neurotoxic mechanism. Here we provide new insights about HO-1 in the brain of subjects with AD and MCI, this latter condition being the transitional phase between normal aging and early AD. HO-1 protein levels were significantly increased in the hippocampus of AD subjects whereas HO-2 protein levels were found significantly decreased in both AD and MCI hippocampi. In addition, significant increases of Ser-residue phosphorylation together with increased oxidative post-translational modifications were found in the hippocampus of AD subjects. Interestingly, despite the lack of oxidative stress-induced AD neuropathology in cerebellum, HO-1 demonstrated increased Ser-residue phosphorylation and oxidative post-translational modifications in this brain area, suggesting HO-1 as a target of oxidative damage even in the cerebellum. The significance of these findings are profound and open new avenues in the comprehension of the role of HO-1 in the pathogenesis of AD.

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Keywords

Alzheimer disease; Heme oxygenase; Mild cognitive impairment; Oxidative stress

Introduction

Increased oxidative and nitrosative stress represents one of the main mechanisms involved in the pathogenesis of neurodegenerative disorders such as Alzheimer disease (AD), which exhibits a large impairment of neuronal structure and molecular pathways due to oxidative stress-induced post-translational modifications on both proteins and lipids [1, 2].

AD is an age-related neurodegenerative disorder characterized histopathologically by the presence of senile plaques (SP), neurofibrillary tangles (NFT), synapse loss in selected brain regions [3, 4], and clinically by memory loss and dementia [5]. The main component of senile plaques is amyloid beta-peptide (A β), a 40–42 amino acid peptide derived by the proteolytic cleavage of amyloid precursor protein (APP) through the activity of beta- and gamma- secretases [4]. Although A β (1-42) is a neurotoxic peptide which exists in both soluble (monomers, oligomers, and protofibrils) and insoluble (fibrils) forms [6], recent studies suggested that the small oligomers, rather than A β fibrils, are the actual toxic species of this peptide [7–10] being responsible of oxidative/nitrosative-induced damage in the brain [1, 11–13]. Amnesic mild cognitive impairment (MCI) is considered the transitional phase between normal aging and early AD [14]. MCI shares with AD both pathological features, such as A β and NFT accumulation in the neocortex and medial temporal lobe [14, 15], which leads to elevated pro-oxidant status [16] and clinical aspects including memory loss [16]. However, MCI, subjects are not characterized by dementia with subjects able to perform normal activities of daily living [17].

Under condition of prolonged oxidative and nitrosative stress brain reacts by up-regulating genes involved in cell stress response processes to limit neuronal damage [18, 19]. The heme oxygenase/biliverdin reductase (HO/BVR) system, whose up-regulation is one of the earlier events in AD, plays a crucial role in the adaptive response to stress [20]. Heme oxygenase is a microsomal enzyme that exists in two main isoforms: the inducible HO-1 and the constitutive HO-2 [21]. Heme oxygenase-1, also known as heat shock protein-32, is induced by various stimuli, including reactive oxygen and nitrogen species (ROS and RNS, respectively), ischemia, heat shock, bacterial lipopolysaccharide (LPS), hemin, and the neuroprotective agent leteprinim potassium (Neotrofin) and is primarily involved in cell stress response [21–23]. Conversely HO-2 is responsive to developmental factors and adrenal glucocorticoids and works as intracellular sensor of oxygen, carbon monoxide and nitric oxide [21, 23]. Furthermore, our group demonstrated an up-regulation of both HO-1 and HO-2 in the brain of aged dogs following atorvastatin treatment [24]. Heme oxygenase catalyzes the oxidation of the alpha-meso-carbon bridge of heme moieties, resulting in equimolar amount of pleiotropic gaseous neuromodulator carbon monoxide (CO), ferrous iron, and biliverdin-IX-alpha. This latter is further reduced by the cytosolic enzyme biliverdin reductase-A (BVR-A) into the powerful antioxidant bilirubin-IX alpha (BR), the final product of heme catabolism [25–27]. It is noteworthy to mention that the activity of both HO-1 and BVR-A was demonstrated to be regulated by the phosphorylation of serine/threonine/tyrosine residues [28, 29].

In the central nervous system (CNS) HO-2 is expressed in neuronal populations in almost all brain areas [21], whereas the inducible isoform is present at low levels in scattered groups of neurons, including the ventromedial and paraventricular nuclei of the hypothalamus [21, 23]. HO-1 is also found in glial cells where its expression can be induced by oxidative stress

[30]. Similarly, BVR-A is co-expressed with HO-1 and/or HO-2 in cells of the rat brain that express these enzymes under normal conditions. BVR-A is also found in regions and cell types that can express heat shock-inducible HO-1 [31].

Recent studies raised the questions about the activation of the HO-1/BVR-A system in neurodegenerative disorders, opening a debate on its real pathophysiological and clinical significance. In particular, lately, our group reported alterations in BVR activity related to decreased phosphorylation and increased oxidative/nitrosative post-translational modifications in the brain of AD and MCI subjects [32, 33]. Furthermore, Hui *et al.*, in a recent work, provided a potential pathway to explain tau aggregation, through a mechanism involving excessive iron production mediated by HO-1 overexpression, which in turn induces tau phosphorylation [34]. In addition, Schipper *et al.* showed that targeted suppression of glial HO-1 hyperactivity may prove to be a rational and effective neurotherapeutic intervention in AD [35]. In this scenario, a deeper level of analysis is required in order to elucidate the contribution of HO/BVR system in neurodegenerative disorders.

Based on the evidence that, despite an up-regulation of HO-1/BVR-A system, a substantial protection against oxidative and nitrosative stress is not observed in AD brain, we hypothesized that, as for BVR-A, even HO-1 could be a target of oxidative/nitrosative stress. The aim of this study was to investigate HO-1 protein levels along with (i) phosphorylation- and (ii) oxidative/nitrosative stress-induced post-translational modifications in both hippocampus and cerebellum of subjects with AD or MCI.

Materials and Methods

Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. Nitrocellulose membranes and electrophoresis transfer system Trans-blot semi-dry Transfer Cell were obtained from Bio-Rad (Hercules, CA, USA). Anti-mouse and anti-rabbit IgG horseradish peroxidase conjugate secondary antibody and ECL plus Western blot detection reagents were obtained from GE Healthcare Bio-Sciences corp. (Piscataway, NJ, USA).

Subjects

Frozen hippocampal and cerebellar samples (n=6 each) from well-characterized subjects with AD and MCI and respective age-matched controls (Table 1) were obtained from the University of Kentucky Rapid Autopsy Program of the Alzheimer's Disease Clinical Center (UK ADC) with a post-mortem interval within the range 1.75–5.75 h for AD and MCI patients and age-matched control subjects. All the subjects were longitudinally followed and underwent annual neuropsychological testing, and neurological and physical examinations. Control subjects were without history of dementia or other neurological disorders and with intact activities of daily living (ADLs), and they underwent annual mental status testing and semi-annual physical and neurological exams as part of the UK ADC normal volunteer longitudinal aging study. The control subjects showed no significant histopathological alterations and the Braak score was within the range I-II for the MCI age-matched controls, and I-III for the AD age-matched controls. Patients diagnosed with MCI met the criteria described by Petersen [14], which included: a memory complaint supported by an informant, objective memory test impairment (age- and education-adjusted), general normal global intellectual function, intact ADLs, Clinical Dementia Rating score of 0.0 to 0.5, no dementia, and a clinical evaluation that revealed no other cause for memory decline. AD patient diagnosis was made according to criteria developed by the National Institute of

Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's disease and Related Disorders Association (ADRD) [36]. All AD patients displayed progressive intellectual decline. The Braak scores were within the range III–V and V–VI for MCI and AD patients, respectively.

Sample preparation

Brain tissues samples (hippocampus and cerebellum) from control, MCI and AD subjects were sonicated in Media 1 lysis buffer (pH 7.4) containing 0.32 M sucrose, 0.10 mM Tris-HCl (pH=8.8), 0.10 mM MgCl₂, 0.08 mM EDTA, proteinase inhibitors leupeptin (0.5 mg/mL), pepstatin (0.7 µg/mL), aprotinin (0.5 mg/mL) and PMSF (40 µg/mL) and phosphatase inhibitor cocktail. Since the phosphorylation of serine residues by specific kinase (e.g. Akt/PKB) are involved in HO-1 activity [28], kinase inhibitors could interfere with such activity. For this reason, kinase inhibitors were not included in Media 1 lysis buffer. Homogenates were centrifuged at 14,000g for 10 min to remove debris. Protein concentration in the supernatant was determined by the Pierce BCA method (Pierce, Rockford, IL, USA).

Western blot analysis

For the evaluation of HO-1 and HO-2 protein levels, 50 µg of total protein of brain homogenate were denatured in sample buffer for 5 min at 100 °C, and then separated on 12% precast Criterion gels (Bio-Rad) by electrophoresis at 100 mA for 2 h in MOPS buffer (Bio-Rad) in a Bio-Rad apparatus. For the evaluation of HO-1 post-translational modifications, 150 µg of total protein of brain homogenate were used, as described below. The proteins from the gels were then transferred to nitrocellulose membrane using the Transblot-Blot SD Semi-Dry Transfer Cell at 20 mA for 2 h. Subsequently, the membranes were blocked at 4 °C for 1 h with fresh blocking buffer made of 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) containing 0.01% (w/v) sodium azide and 0.2% (v/v) Tween 20 (PBST). The membranes were incubated at room temperature in PBST for 2 h with the following primary antibodies, as separate experiments: anti-HO-1, (Stressgen, Ann Arbor, MI, USA, dilution 1:1000); anti-HO-2 (Stressgen, Ann Arbor, MI, USA, dilution 1:1000) anti-phosphoserine (Zymed, Invitrogen, Camarillo, CA, USA, dilution 1:250), anti-phosphothreonine (Zymed, Invitrogen, Camarillo, CA, USA, dilution 1:250); anti-phosphotyrosine, (Zymed, Invitrogen, Camarillo, CA, USA, dilution 1:1000); anti-nitrotyrosine (3-NT) (Sigma-Aldrich, dilution 1:100), anti-dinitrophenylhydrazine (DNP) protein adducts (Millipore, Billerica, MA, USA, dilution 1:100), anti-4-hydroxy-2-nonenal (HNE) (Alpha Diagnostic International, San Antonio, TX, USA, dilution 1:100) and anti-β-actin (Sigma-Aldrich, dilution 1:2000). The membranes were then washed three times for 5 min with PBST followed by incubation with anti-mouse alkaline phosphatase or horseradish peroxidase conjugate secondary antibody (1:3000) in PBST for 2 h at room temperature. Membranes were then washed three times in PBST for 5 min and developed using or 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium (BCIP/NBT) color developing reagent for alkaline phosphatase secondary antibody or ECL plus WB detection reagents for horseradish peroxidase conjugate secondary antibody. Blots were dried, scanned in TIF format using Adobe Photoshop on a Canoscan 8800F (Canon) or STORM UV transilluminator (λ_{ex} = 470 nm, λ_{em} = 618 nm, Molecular Dynamics, Sunnyvale, CA, USA) for chemiluminescence. The images were quantified with Image Quant TL 1D version 7.0 software (GE Healthcare). The optical density of bands was calculated as volume (optical density × area) adjusted for the background.

Immunoprecipitation

The immunoprecipitation procedure was performed as previously described [37] with modifications. Briefly, 150 µg of protein extracts were dissolved in 500 µl of RIPA buffer (10 mM Tris, pH 7.6; 140 mM NaCl; 0.5% NP40 including protease inhibitors) and then

incubated with 1 µg of anti-HO-1 antibody at 4°C overnight. Immunocomplexes were collected by using protein A/G suspension for 2 h at 4°C and washed five times with immunoprecipitation buffer. Immunoprecipitated HO-1 was recovered by resuspending the pellets in reducing SDS buffers and subjected to electrophoresis on 12% gels followed by Western blot analysis. The membranes were then stripped and re-probed using an anti-HO-1 antibody as described above. Total HO-1 was used as loading control according to [38, 39].

Post-derivatization of protein

Samples were post-derivatized with dinitrophenylhydrazine (DNPH) on the membrane and probed with anti-DNP antibody to identify the carbonylated proteins. The nitrocellulose membranes were equilibrated in solution A (20% (v/v) methanol and 80% (v/v) wash blot buffer [phosphate- buffered saline (PBS) solution containing 0.04% (v/v) Tween 20 and 0.10 M NaCl]) for 5 min, followed by incubation of membranes in 2N HCl for 5 min. The proteins on blots were then derivatized in solution B (0.5 mM DNPH in 2N HCl) for 10 min as described by [40]. The membranes were successively washed 5 min per time in 2N HCl for three times, wash blot buffer/methanol (50/50) for five times and finally wash blot buffer for two times. The DNP adducts were detected immunochemically as described above.

Statistical analysis

All statistical analysis was performed using a two-tailed Student's t-test. $P < 0.05$ was considered significantly different from control.

Results

HO-1 and HO-2 protein levels in hippocampus and cerebellum of subjects with AD or MCI

In 1995 Shipper and colleagues observed intense immunoreactivity of HO-1 in neurons of the hippocampus and temporal cortex of Alzheimer-diseased (AD) brain relative to age-matched control specimens [41]. In addition, we previously observed an increased expression of HO-1 together with a decreased expression of HO-2 in the inferior parietal lobule of AD brains, a region that showed elevated oxidative and nitrosative stress [42].

Before proceeding with the analysis of post-translational modifications, we first analyzed HO-1 and HO-2 protein levels in our samples. In the hippocampus of AD subjects, HO-1 protein levels are significantly increased by 119% with respect to aged matched controls (Figure 1a). Conversely, HO-2 protein levels are significantly decreased by 35% in the same brain area (Figure 1b). With respect to MCI, a previous study from our group performed by using the same set of samples, showed that HO-1 protein levels, in both hippocampus and cerebellum, were not significantly different to those observed in aged matched controls [43]. Here we extended the analysis to HO-2 protein levels, which show a significant decrease by about 30% in the hippocampus of subjects with MCI (Figure 1c). In cerebellum, HO-1 protein levels were decreased (~38%) in AD subjects with respect to the matched controls (Figure 1d), although this value did not reach statistical significance. No significant differences were found for HO-2 in the same brain area (Figure 1e). Finally, no changes are observed for HO-2 protein levels in the cerebellum of MCI subjects with respect to the matched controls (Figure 2f).

HO-1 phosphorylation modifications in the hippocampus and cerebellum of subjects with AD or MCI

In 2004 Salinas *et al.* demonstrated that human HO-1 can be phosphorylated at specific serine residues and such phosphorylation regulates the interaction with BVR as well as its own activity [28]. In contrast HO-2 has a non-phosphorylatable arginine instead of Ser [28]. For this reason, and due to the significant decrease of protein levels observed in AD and

MCI hippocampi, HO-2 was not taken into account for the analysis of post-translational modifications. In order to clarify if HO-1 undergoes phosphorylation modifications in the hippocampus and cerebellum of subjects with AD or MCI, we analyzed the levels of phospho-serine (pSer), phospho-threonine (pThr) and phospho-tyrosine (pTyr) of HO-1. As shown in Figure 2a, pSer-HO-1 is significantly increased in the hippocampus of subjects with AD, whereas no changes were observed for pThr-HO-1 (Figure 2b). Interestingly, no signal was obtained for pTyr-HO-1 (Figure 2c), probably due to the lower number of Tyr residues in HO-1 with respect to Ser and Thr residues [44], or to a lower involvement of Tyr phosphorylation. In the hippocampus of subjects with MCI no significant differences were observed for both pSer-HO-1 (Figure 2d) and pThr-HO-1 (Figure 2e) with respect to the matched controls. As for AD, no signal was obtained as regard the analysis of pTyr-HO-1 in the hippocampus of subjects with MCI (data not shown). Finally, to evaluate whether or not changes observed for pSer-HO-1 levels were specific for hippocampal tissue, the same experiments were performed in the cerebellum. pSer-HO-1 is significantly increased by 21% (Figure 3a) and 24% (Figure 3b) in the cerebellum of subjects with AD or MCI, respectively.

Protein carbonyls (PC), protein-bound 4-hydroxy-2-nonenal (HNE) and 3-nitrotyrosine (3-NT) levels on HO-1 in the hippocampus and cerebellum of subjects with AD or MCI

To evaluate if HO-1 is a target for oxidative and nitrosative stress-induced post-translational modifications, the levels of PC, protein-bound HNE and 3-NT on HO-1 were evaluated in the hippocampus and cerebellum of subjects with AD or MCI. PC (Figure 4a) and protein-bound HNE (Figure 4b) on HO-1 were significantly increased by 30% and 52% respectively, whereas no signal detection was observed for 3-NT (Figure 4c) in the hippocampus of subjects with AD compared to aged-matched controls. Together with the absence of pTyr-HO-1 signal detection, this last evidence likely is involved in the smaller contribution of Tyr residues in HO-1 post-translational modifications. Interestingly, in the hippocampus of subjects with MCI no changes were found for PC (Figure 4d), whereas a significant increase of 85%, was observed in the levels of HNE-bound HO-1 (Figure 4e). The analysis of cerebellar samples revealed no changes for the levels of PC in HO-1 on AD subjects with respect to the matched controls (Figure 5a), while a significant increase of 36% of HNE-bound HO-1 (Figure 5b) was observed. In contrast, in the cerebellum from subjects with MCI, we observed significant increased levels of PC (about 32%) on HO-1 (Figure 5c), whereas no changes were demonstrated for levels of HNE-bound HO-1 (Figure 5d).

Discussion

Since it was discovered, the HO-1/BVR-A system was considered a useful mechanism through which cells respond to oxidative/nitrosative stress insults, in order to prevent the impairment of cellular homeostasis. However, the effective contribution of HO-1/BVR-A system induction to cellular antioxidant defense is currently under debate because a growing number of evidences questioned its protective role in neurodegenerative disorders.

In this paper our primary goal was to determine if evidence existed about HO-1 post-translational modifications in the brain of AD and MCI subjects. We chose to investigate hippocampus and cerebellum brain areas for this study because of their differential involvement in free radical-induced injury and pathology. Indeed, hippocampus is broadly recognized as a main target of neurodegenerative damage during AD progression, presenting increased levels of oxidative stress, neuronal loss and marked atrophy with respect to the whole brain. Conversely, cerebellum is largely devoid of pathology and oxidative stress [45–47].

The first novel result provided by the current study is the different profiles of HO-1 and HO-2 in the brain of subjects with AD or MCI. Previous data from Premkumar and colleagues have already shown a different pattern of expression for HO-1 and HO-2 among neocortex, cerebellum and cerebral vessel from AD subjects [48]. Here we extend the neurobiological behavior of 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. The increase of HO-1 in the hippocampus of AD subjects (Figure 1a) is in good agreement with previous results [41, 48], and could be easily explained by the elevated levels of oxidative stress observed in this brain area [21], while the decrease of HO-2 protein levels (Figure 1b and 1c) is less obvious and represents an intriguing finding. Very few lines of evidence exist about the possibility to modulate HO-2 expression [24, 49, 50]. In the present case we speculate that one possible explanation could come from the link between HO-2 and glucocorticoids. As previously demonstrated, the HO-2 gene possesses in the promoter region a consensus sequence of the glucocorticoid response element (GRE), which plays a main role in glucocorticoid-induced changes in HO-2 protein levels [51]. In 1994, Weber and colleagues demonstrated that corticosterone treatment decreased HO-2 protein levels in the hippocampus [49]. Furthermore, more recently, Chen *et al.* demonstrated that chronic restraint stress decreased HO-2 protein levels in hippocampal neurons and this stress-induced decrease in HO-2 protein levels may result from high corticosterone levels [50]. In addition, an impairment of the hypothalamic–pituitary–adrenal (HPA) axis, which triggers the adrenal cortex to release glucocorticoids (cortisol in primates, corticosterone in mice and rats), was associated with AD and MCI pathogenesis [52–54]. In fact, glucocorticoids have been suggested not only to contribute to age-related loss of neurons in the hippocampus of rats [55], but was also shown to potentiate hippocampal damage induced by various noxious insults [56], including A β peptide [57]. Thus, a conceivable justification about the significant decrease of HO-2 protein levels observed in the hippocampus of both AD and MCI subjects could be represented by corticosterone-induced decrease of the protein in this brain area [49].

With regard to HO-1, an interesting aspect of our work is the observed significant increase of Ser-residue phosphorylation along with oxidative post-translational modifications in the hippocampus of AD subjects (Figures 2a, 4b and 4c). Since HO-1 is a stress-inducible protein, and phosphorylation on Ser residues appears to be important for its activation [28], 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 [28]. At the same time, the increased oxidative stress could be responsible for the observed rise of PC and HNE-adducts, as well as was demonstrated for other proteins in AD [16], including BVR-A [32, 33], leading to altered protein structure and function impairment [11, 58–60]. 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 HO-1 oxidative damage (e.g., increased PC and HNE-adducts on its structure). Consequently, the cell tries to restore the functionality of the protein by increasing Ser residues 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 (Figure 6). The finding of the presence of either oxidative (HNE) and phosphorylative (pSer) modifications on HO-1 brings to mind the past results by Takeda *et al.* about tau conformational changes in the hippocampus of AD subjects [61]. In particular, these authors showed that the increase of both HNE production and tau-phosphorylation are two events needed for the promotion of tau conformational changes which in turn are

associated with the induction of HO-1 in the same neurons [61]. These data, together with those by Hui et al. showing that overexpression HO-1 promote tau phosphorylation and aggregation [34], provide new insights for understanding the mechanisms involved in tau-pathology. In our opinion, they refer of a tight link between HO-1 and tau, in which, as in a vicious circle, HO-1 could be induced in response to tau phosphorylation and oxidative stress in order to protect the neurons [61], but at the same time, if over-expressed it could trigger tau aggregation and toxicity [34]. However, in the above cited reports, the only parameter taken into account were the expression levels of HO-1, thus it is not completely clear if the mechanisms proposed are linked to a specific signal-transduction pathway mediated by HO-1. It should be very interesting in the future to evaluate if, based on our results, the post-translational modifications on HO-1 observed in AD and MCI brain could affect tau-pathology positively or negatively.

With regard to MCI, the results from hippocampus add new elements to the comprehension of the contribution of the HO-1/BVR-A system to AD pathogenesis. Unlike BVR-A, whose expression levels were found significantly increased even in the hippocampus of subjects with MCI [33], HO-1 protein levels do not present any differences [43]. This result could mean that the induction of each member of HO-1/BVR-A system is not concomitant and probably the threshold levels of oxidative/nitrosative stress needed to induce HO-1 and BVR-A are different. Due to the pleiotropic functions of BVR-A in the maintenance of cellular homeostasis [29], it is plausible that the induction of BVR-A precedes those of HO-1. On the contrary, the formation of HNE-adducts on HO-1 (Figure 4e), along with BVR-A nitration [32], are already evident in the hippocampus of subjects with MCI. In this light, despite the progressive increase of HO-1/BVR-A protein levels observed from MCI to AD [62], the impairment of the system likely is an early event in the pathogenesis and progression of the disease. A different behavior is shown for protein phosphorylation. We did not observe any significant difference in pSer-HO-1 in the hippocampus of MCI subjects with respect to aged matched controls (Figure 2d), suggesting that the increase of phosphorylation process occurs in a later stage of the disease.

We can speculate that increased Ser-residue phosphorylation along with increased protein levels could act as a compensatory mechanism to overcome the inactivation of HO-1 by oxidative damage. However, whether or not HO-1 functionality is in part restored after Ser-residue phosphorylation remains an unsolved question. In order to complete this intricate puzzle, the measure of HO-1 activity should be considered. However, it is not possible to single out the differential contribution of HO-1 and HO-2 to the generation of their products (i.e., CO, ferrous iron and biliverdin) due to lack of reliable selective inhibitors [27].

Another problem involves the possible repercussions of HO-1 up-regulation on cell metabolism. As noted earlier, with respect to metabolism, HO activity is something of a double-edged sword [63, 64]. The reduced availability of heme after HO-catalyzed degradation may provide useful antioxidant effects, but prolonged HO activation could produce low enough intracellular heme levels incapable of meeting the cell's metabolic requirements [63, 64]. In fact, heme is the prosthetic group of hemoglobin, myoglobin, cytochromes, and several other important proteins, and excessively low concentrations can impair mitochondrial function and cell respiration. Furthermore, both CO and iron, which have physiological effects when produced under basal conditions, may become toxic if produced in excess [65–67].

In the scenario described above, these considerations are in good agreement with the concerns about the neuroprotective or neurotoxic role of HO-1 in AD: i) The failure to protect neurons against the deleterious effects of oxidative/nitrosative stress could be due to an impairment of HO-1, together with BVR-A, as suggested by our group [32, 33]; ii)

Phosphorylation is able to restore HO-1 functionality and as a consequence the sustained activation of HO-1 could be responsible, at least in part, for the observed increased oxidative stress, as well as tau phosphorylation, in the hippocampus of AD subjects, as suggested by other groups [34, 35]. Thus, we suggest that the neuroprotective effects mediated by the HO/BVR-A system can be obtained only if the fine balance between the activity of HO-1 or HO-2 and that of BVR-A are maintained.

Another novel finding of the present study regards the cerebellum, which has long been considered as an internal control due to minimal neuropathology [46]. Our results show a significant increase of HO-1 oxidation in the cerebellum of both MCI and AD subjects (Figure 5b and 5c), although the elevations of HO-1 oxidation levels in a brain region with limited neuropathology may appear contradictory. However, recent studies suggest that disease-related changes could occur even in this brain area [68–70]. Furthermore, an increase of neurotoxic markers of lipid peroxidation (HNE or acrolein) and iron dyshomeostasis were found at an early stage of the disease such as MCI or pre-clinical AD [46, 70]. Thus, HO-1 could represent a likely target of oxidative damage even in the cerebellum. Finally, the observed increase of Ser-residue phosphorylation (Figure 3a and 3b) along with oxidative modification in the cerebellum of MCI and AD subjects is in agreement with the explanation given for the hippocampus.

Conclusion

In conclusion, our previous studies coupled with the current investigation show that the HO-1/BVR-A system is impaired in AD and MCI brain. In our opinion, it is no longer correct to measure only total HO-1 or BVR-A 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. The significance of these lines of evidence is profound, and *ad hoc* research to clarify the mechanisms involved in the regulation of HO-1/BVR-A system in AD and MCI are ongoing in our laboratory.

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Abbreviations

3-NT	3-nitrotyrosine
AD	Alzheimer disease
HNE	4-hydroxy-2-nonenal
HO	heme oxygenase
MCI	mild cognitive impairment
PC	protein carbonyls
pSer	phosphoserine
pThr	phosphothreonine
pTyr	phosphotyrosine

References

1. Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. *Free radical biology & medicine*. 1997; 23:134–147. [PubMed: 9165306]
2. Smith MA, Richey PL, Taneda S, Kutty RK, Sayre LM, Monnier VM, Perry G. Advanced Maillard reaction end products, free radicals, and protein oxidation in Alzheimer's disease. *Ann N Y Acad Sci*. 1994; 738:447–454. [PubMed: 7832455]
3. Mirra SS. The CERAD neuropathology protocol and consensus recommendations for the postmortem diagnosis of Alzheimer's disease: a commentary. *Neurobiol Aging*. 1997; 18:S91–94. [PubMed: 9330994]
4. Querfurth HW, LaFerla FM. Alzheimer's disease. *N Engl J Med*. 2010; 362:329–344. [PubMed: 20107219]
5. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. *Lancet*. 2011; 377:1019–1031. [PubMed: 21371747]
6. Mohamed A, Cortez L, de Chaves EP. Aggregation state and neurotoxic properties of alzheimer beta-amyloid peptide. *Curr Protein Pept Sci*. 2011; 12:235–257. [PubMed: 21348837]
7. Drake J, Link CD, Butterfield DA. Oxidative stress precedes fibrillar deposition of Alzheimer's disease amyloid beta-peptide (1-42) in a transgenic *Caenorhabditis elegans* model. *Neurobiol Aging*. 2003; 24:415–420. [PubMed: 12600717]
8. Lambert JC, Mann DM, Harris JM, Chartier-Harlin MC, Cumming A, Coates J, Lemmon H, StClair D, Iwatsubo T, Lendon C. The -48 C/T polymorphism in the presenilin 1 promoter is associated with an increased risk of developing Alzheimer's disease and an increased A β load in brain. *J Med Genet*. 2001; 38:353–355. [PubMed: 11389157]
9. Oda T, Wals P, Osterburg HH, Johnson SA, Pasinetti GM, Morgan TE, Rozovsky I, Stine WB, Snyder SW, Holzman TF, et al. Clusterin (apoJ) alters the aggregation of amyloid beta-peptide (A β 1-42) and forms slowly sedimenting A β complexes that cause oxidative stress. *Exp Neurol*. 1995; 136:22–31. [PubMed: 7589331]
10. Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, Benedek GB, Selkoe DJ, Teplow DB. Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J Biol Chem*. 1999; 274:25945–25952. [PubMed: 10464339]
11. Butterfield DA, Lauderback CM. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid beta-peptide-associated free radical oxidative stress. *Free radical biology & medicine*. 2002; 32:1050–1060. [PubMed: 12031889]
12. Smith MA, Perry G, Pryor WA. Causes and consequences of oxidative stress in Alzheimer's disease. *Free radical biology & medicine*. 2002; 32:1049. [PubMed: 12031888]
13. Butterfield DA, Galvan V, Lange MB, Tang H, Sowell RA, Spilman P, Fombonne J, Gorostiza O, Zhang J, Sultana R, Bredesen DE. In vivo oxidative stress in brain of Alzheimer disease transgenic mice: Requirement for methionine 35 in amyloid beta-peptide of APP. *Free Radic Biol Med*. 2010; 48:136–144. [PubMed: 19854267]
14. Petersen RC. Mild cognitive impairment clinical trials. *Nat Rev Drug Discov*. 2003; 2:646–653. [PubMed: 12904814]
15. Jicha GA, Parisi JE, Dickson DW, Johnson K, Cha R, Ivnik RJ, Tangalos EG, Boeve BF, Knopman DS, Braak H, Petersen RC. Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. *Arch Neurol*. 2006; 63:674–681. [PubMed: 16682537]
16. Sultana R, Perluigi M, Butterfield DA. Oxidatively modified proteins in Alzheimer's disease (AD), mild cognitive impairment and animal models of AD: role of A β in pathogenesis. *Acta Neuropathol*. 2009; 118:131–150. [PubMed: 19288120]
17. Petersen RC. Clinical practice. Mild cognitive impairment. *N Engl J Med*. 2011; 364:2227–2234. [PubMed: 21651394]
18. Calabrese V, Cornelius C, Mancuso C, Barone E, Calafato S, Bates T, Rizzarelli E, Kostova AT. Vitagenes, dietary antioxidants and neuroprotection in neurodegenerative diseases. *Frontiers in bioscience: a journal and virtual library*. 2009; 14:376–397. [PubMed: 19273073]
19. Mancuso C, Scapagini G, Curro D, Giuffrida Stella AM, De Marco C, Butterfield DA, Calabrese V. Mitochondrial dysfunction, free radical generation and cellular stress response in

- neurodegenerative disorders. *Frontiers in bioscience: a journal and virtual library*. 2007; 12:1107–1123. [PubMed: 17127365]
20. Poon HF, Calabrese V, Scapagnini G, Butterfield DA. Free radicals: key to brain aging and heme oxygenase as a cellular response to oxidative stress. *J Gerontol A Biol Sci Med Sci*. 2004; 59:478–493. [PubMed: 15123759]
 21. Maines MD. The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol*. 1997; 37:517–554. [PubMed: 9131263]
 22. Maines MD. The heme oxygenase system and its functions in the brain. *Cell Mol Biol (Noisy-le-grand)*. 2000; 46:573–585. [PubMed: 10872744]
 23. Mancuso C. Heme oxygenase and its products in the nervous system. *Antioxid Redox Signal*. 2004; 6:878–887. [PubMed: 15345148]
 24. Butterfield DA, Barone E, Di Domenico F, Cenini G, Sultana R, Murphy MP, Mancuso C, Head E. Atorvastatin treatment in a dog preclinical model of Alzheimer's disease leads to up-regulation of haem oxygenase-1 and is associated with reduced oxidative stress in brain. *Int J Neuropsychopharmacol*. 2011 in press. 10.1017/S1461145711001118
 25. Maines MD, Panahian N. The heme oxygenase system and cellular defense mechanisms. Do HO-1 and HO-2 have different functions? *Adv Exp Med Biol*. 2001; 502:249–272. [PubMed: 11950143]
 26. Stocker R. Antioxidant activities of bile pigments. *Antioxid Redox Signal*. 2004; 6:841–849. [PubMed: 15345144]
 27. Mancuso C, Barone E. The heme oxygenase/biliverdin reductase pathway in drug research and development. *Curr Drug Metab*. 2009; 10:579–594. [PubMed: 19702533]
 28. Salinas M, Wang J, Rosa de Sagarra M, Martin D, Rojo AI, Martin-Perez J, Ortiz de Montellano PR, Cuadrado A. Protein kinase Akt/PKB phosphorylates heme oxygenase-1 in vitro and in vivo. *FEBS Lett*. 2004; 578:90–94. [PubMed: 15581622]
 29. Kapitulnik J, Maines MD. Pleiotropic functions of biliverdin reductase: cellular signaling and generation of cytoprotective and cytotoxic bilirubin. *Trends Pharmacol Sci*. 2009; 30:129–137. [PubMed: 19217170]
 30. 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. [PubMed: 7609642]
 31. 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. [PubMed: 8360669]
 32. Barone E, Di Domenico F, Cenini G, Sultana R, Coccia R, Preziosi P, Perluigi M, Mancuso C, Butterfield DA. Oxidative and nitrosative modifications of biliverdin reductase-A in the brain of subjects with Alzheimer's disease and amnesic mild cognitive impairment. *Journal of Alzheimer's disease: JAD*. 2011; 25:623–633.
 33. Barone E, Di Domenico F, Cenini G, Sultana R, Cini C, Preziosi P, Perluigi M, Mancuso C, Butterfield DA. Biliverdin reductase--a protein levels and activity in the brains of subjects with Alzheimer disease and mild cognitive impairment. *Biochimica et biophysica acta*. 2011; 1812:480–487. [PubMed: 21241799]
 34. Hui Y, Wang D, Li W, Zhang L, Jin J, Ma N, Zhou L, Nakajima O, Zhao W, Gao X. Long-term overexpression of heme oxygenase 1 promotes tau aggregation in mouse brain by inducing tau phosphorylation. *J Alzheimers Dis*. 2011; 26:299–313. [PubMed: 21613741]
 35. Schipper HM, Gupta A, Szarek WA. Suppression of glial HO-1 activity as a potential neurotherapeutic intervention in AD. *Curr Alzheimer Res*. 2009; 6:424–430. [PubMed: 19874266]
 36. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984; 34:939–944. [PubMed: 6610841]
 37. Cenini G, Sultana R, Memo M, Butterfield DA. Effects of oxidative and nitrosative stress in brain on p53 proapoptotic protein in amnesic mild cognitive impairment and Alzheimer disease. *Free Radic Biol Med*. 2008; 45:81–85. [PubMed: 18439434]

38. Salim M, Brown-Kipphut BA, Maines MD. Human biliverdin reductase is autophosphorylated, and phosphorylation is required for bilirubin formation. *J Biol Chem.* 2001; 276:10929–10934. [PubMed: 11278740]
39. Lerner-Marmarosh N, Miralem T, Gibbs PE, Maines MD. Human biliverdin reductase is an ERK activator; hBVR is an ERK nuclear transporter and is required for MAPK signaling. *Proc Natl Acad Sci U S A.* 2008; 105:6870–6875. [PubMed: 18463290]
40. Conrad CC, Talent JM, Malakowsky CA, Gracy RW. Post-Electrophoretic Identification of Oxidized Proteins. *Biol Proced Online.* 2000; 2:39–45. [PubMed: 12734585]
41. Schipper HM, Cisse S, Stopa EG. Expression of heme oxygenase-1 in the senescent and Alzheimer-diseased brain. *Ann Neurol.* 1995; 37:758–768. [PubMed: 7778849]
42. Calabrese V, Sultana R, Scapagnini G, Guagliano E, Sapienza M, Bella R, Kanski J, Pennisi G, Mancuso C, Stella AM, Butterfield DA. Nitrosative stress, cellular stress response, and thiol homeostasis in patients with Alzheimer's disease. *Antioxid Redox Signal.* 2006; 8:1975–1986. [PubMed: 17034343]
43. Di Domenico F, Sultana R, Tiu GF, Scheff NN, Perluigi M, Cini C, Butterfield DA. Protein levels of heat shock proteins 27, 32, 60, 70, 90 and thioredoxin-1 in amnesic mild cognitive impairment: an investigation on the role of cellular stress response in the progression of Alzheimer disease. *Brain Res.* 2010; 1333:72–81. [PubMed: 20362559]
44. Maines MD. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. *FASEB J.* 1988; 2:2557–2568. [PubMed: 3290025]
45. Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, Markesbery WR. Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology.* 2005; 64:1152–1156. [PubMed: 15824339]
46. Hensley K, Hall N, Subramaniam R, Cole P, Harris M, Aksenov M, Aksenova M, Gabbita SP, Wu JF, Carney JM, et al. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. *J Neurochem.* 1995; 65:2146–2156. [PubMed: 7595501]
47. Markesbery WR. Neuropathologic alterations in mild cognitive impairment: a review. *J Alzheimers Dis.* 2010; 19:221–228. [PubMed: 20061641]
48. Premkumar DR, Smith MA, Richey PL, Petersen RB, Castellani R, Kutty RK, Wiggert B, Perry G, Kalaria RN. Induction of heme oxygenase-1 mRNA and protein in neocortex and cerebral vessels in Alzheimer's disease. *Journal of neurochemistry.* 1995; 65:1399–1402. [PubMed: 7543935]
49. 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. [PubMed: 7519667]
50. Chen Z, Xu H, Haimano S, Li X, Li XM. Quetiapine and venlafaxine synergically regulate heme oxygenase-2 protein expression in the hippocampus of stressed rats. *Neurosci Lett.* 2005; 389:173–177. [PubMed: 16115734]
51. 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. [PubMed: 9116047]
52. Rauchs G, Harand C, Bertran F, Desgranges B, Eustache F. Sleep and episodic memory: a review of the literature in young healthy subjects and potential links between sleep changes and memory impairment observed during aging and Alzheimer's disease. *Rev Neurol (Paris).* 2010; 166:873–881. [PubMed: 20447667]
53. Green KN, Billings LM, Roozendaal B, McGaugh JL, LaFerla FM. Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. *J Neurosci.* 2006; 26:9047–9056. [PubMed: 16943563]
54. Lei JK. Change of serum ACTH and cortisol levels in Alzheimer disease and mild cognition impairment. *Zhonghua Yi Xue Za Zhi.* 2010; 90:2894–2896. [PubMed: 21211392]
55. Sapolsky RM. Glucocorticoid toxicity in the hippocampus: temporal aspects of neuronal vulnerability. *Brain Res.* 1985; 359:300–305. [PubMed: 4075151]
56. Sapolsky RM, Pulsinelli WA. Glucocorticoids potentiate ischemic injury to neurons: therapeutic implications. *Science.* 1985; 229:1397–1400. [PubMed: 4035356]

57. Catania C, Sotiropoulos I, Silva R, Onofri C, Breen KC, Sousa N, Almeida OF. The amyloidogenic potential and behavioral correlates of stress. *Mol Psychiatry*. 2009; 14:95–105. [PubMed: 17912249]
58. Subramaniam R, Roediger F, Jordan B, Mattson MP, Keller JN, Waeg G, Butterfield DA. The lipid peroxidation product, 4-hydroxy-2-trans-nonenal, alters the conformation of cortical synaptosomal membrane proteins. *Journal of neurochemistry*. 1997; 69:1161–1169. [PubMed: 9282939]
59. Owen JB, Sultana R, Aluise CD, Erickson MA, Price TO, Bu G, Banks WA, Butterfield DA. Oxidative modification to LDL receptor-related protein 1 in hippocampus from subjects with Alzheimer disease: implications for Abeta accumulation in AD brain. *Free Radic Biol Med*. 2010; 49:1798–1803. [PubMed: 20869432]
60. Lauderback CM, Hackett JM, Huang FF, Keller JN, Szweda LI, Markesbery WR, Butterfield DA. The glial glutamate transporter, GLT-1, is oxidatively modified by 4-hydroxy-2-nonenal in the Alzheimer's disease brain: the role of Abeta1-42. *J Neurochem*. 2001; 78:413–416. [PubMed: 11461977]
61. Takeda A, Smith MA, Avila J, Nunomura A, Siedlak SL, Zhu X, Perry G, Sayre LM. In Alzheimer's disease, heme oxygenase is coincident with Alz50, an epitope of tau induced by 4-hydroxy-2-nonenal modification. *Journal of neurochemistry*. 2000; 75:1234–1241. [PubMed: 10936206]
62. Barone E, Mancuso C, Di Domenico F, Sultana R, Murphy MP, Head E, Butterfield DA. Biliverdin Reductase-A: a Novel Drug Target for Atorvastatin in a Dog Preclinical Model of Alzheimer Disease. *J Neurochem*. 2011
63. Shibahara S. The heme oxygenase dilemma in cellular homeostasis: new insights for the feedback regulation of heme catabolism. *The Tohoku journal of experimental medicine*. 2003; 200:167–186. [PubMed: 14580148]
64. Shibahara S, Nakayama M, Kitamuro T, Uono-Fujimori R, Takahashi K. Repression of heme oxygenase-1 expression as a defense strategy in humans. *Exp Biol Med (Maywood)*. 2003; 228:472–473. [PubMed: 12709571]
65. Brown SD, Piantadosi CA. Recovery of energy metabolism in rat brain after carbon monoxide hypoxia. *The Journal of clinical investigation*. 1992; 89:666–672. [PubMed: 1737854]
66. Zhang J, Piantadosi CA. Mitochondrial oxidative stress after carbon monoxide hypoxia in the rat brain. *The Journal of clinical investigation*. 1992; 90:1193–1199. [PubMed: 1328293]
67. Schipper HM, Bernier L, Mehindate K, Frankel D. Mitochondrial iron sequestration in dopamine-challenged astroglia: role of heme oxygenase-1 and the permeability transition pore. *Journal of neurochemistry*. 1999; 72:1802–1811. [PubMed: 10217256]
68. Williams TI, Lynn BC, Markesbery WR, Lovell MA. Increased levels of 4-hydroxynonenal and acrolein, neurotoxic markers of lipid peroxidation, in the brain in Mild Cognitive Impairment and early Alzheimer's disease. *Neurobiol Aging*. 2006; 27:1094–1099. [PubMed: 15993986]
69. Bradley MA, Xiong-Fister S, Markesbery WR, Lovell MA. Elevated 4-hydroxyhexenal in Alzheimer's disease (AD) progression. *Neurobiol Aging*. 2010 in press. 10.1016/j.neurobiolaging.2010.1008.1016
70. Smith MA, Zhu X, Tabaton M, Liu G, McKeel DW Jr, Cohen ML, Wang X, Siedlak SL, Dwyer BE, Hayashi T, Nakamura M, Nunomura A, Perry G. Increased iron and free radical generation in preclinical Alzheimer disease and mild cognitive impairment. *J Alzheimers Dis*. 2010; 19:363–372. [PubMed: 20061651]

Research highlights

- Increased heme oxygenase-1(HO-1) expression in Alzheimer disease (AD) hippocampus
- Increased phosphorylation (pSer) on HO-1 in AD hippocampus
- Increased protein carbonyls (PC) and 4-hydroxy-2-nonenal (HNE) on HO-1 in AD hippocampus
- Increased HNE on HO-1 in mild cognitive impairment (MCI) hippocampus
- Results indicate that HO-1 is a target of oxidative modification early in progression of AD

Figure 1. Heme oxygenase-1 (HO-1) and heme oxygenase-2 (HO-2) protein levels in the hippocampus and cerebellum of subjects with Alzheimer disease (AD) and mild cognitive impairment (MCI)

HO-1 protein levels in the hippocampus of AD subjects (a); HO-2 protein levels in the hippocampus of AD (b) and MCI (c) subjects; HO-1 protein levels in the cerebellum of AD subjects (d); HO-2 protein levels in the cerebellum of AD (e) and MCI (f) subjects. Representative gels are shown. Data are expressed as mean $\pm$ SD (n=6 individual samples per group). *P < 0.05 and **P < 0.01 versus control.

Figure 2. Heme oxygenase-1 (HO-1) phosphorylation on Serine (pSer), Threonine (pThr) and Tyrosine (pTyr) residues in the hippocampus of subjects with Alzheimer disease (AD) and mild cognitive impairment (MCI)

pSer (a), pThr (b) and pTyr (c) levels on HO-1 in the hippocampus of AD subjects. pSer (d) and pThr (e) levels on HO-1 in the hippocampus of MCI subjects. Representative gels are shown. Data are expressed as mean $\pm$ SD (n=6 individual samples per group). **P < 0.01 versus control.

Figure 3. Heme oxygenase-1 (HO-1) phosphorylation on Serine (pSer) residues in the cerebellum of subjects with Alzheimer disease (AD) and mild cognitive impairment (MCI)
pSer levels on HO-1 in the cerebellum of AD (a) and MCI (b) subjects. Representative gels are shown. Data are expressed as mean $\pm$ SD (n=6 individual samples per group). *P <0.05 versus control.

Figure 4. Heme oxygenase-1 (HO-1) oxidative and nitrosative post-translational modifications in the hippocampus of subjects with Alzheimer disease (AD) and mild cognitive impairment (MCI) Protein carbonyls (PC) (a), 4-hydroxy-2-nonenals (HNE) (b) and 3-nitrotyrosine (3-NT) (c) levels on HO-1 in the hippocampus of AD subjects. PC (d) and HNE (b) levels on HO-1 in the hippocampus of MCI subjects. Representative gels are shown. Data are expressed as mean $\pm$ SD (n=6 individual samples per group). *P < 0.05 and **P < 0.01 versus control.

Figure 5. Heme oxygenase-1 (HO-1) oxidative and nitrosative post-translational modifications in the cerebellum of subjects with Alzheimer disease (AD) and mild cognitive impairment (MCI) Protein carbonyls (PC) (a) and 4-hydroxy-2-nonenals (HNE) (b) levels on HO-1 in the cerebellum of AD subjects. PC (d) and HNE (b) levels on HO-1 in the cerebellum of MCI subjects. Representative gels are shown. Data are expressed as mean $\pm$ SD (n=6 individual samples per group). *P < 0.05 versus control.

Figure 6. Putative scenario of the mechanisms leading to heme oxygenase-1 (HO-1) post-translational modifications due to increased oxidative stress environment in Alzheimer disease (AD) hippocampus

Blue arrows (1), the increase of oxidative stress levels observed in the hippocampus of AD subjects promotes the increase of HO-1 oxidative damage (e.g., increase of PC and HNE-adducts on its structure). Consequently, the cell tries to restore the functionality of HO-1 by increasing Ser-residue phosphorylation; *Yellow arrows (2)*, the increase of oxidative stress promotes the increase of Ser phosphorylation in order to activate protein functions, then HO-1 quickly becomes a target of oxidative post-translational modifications, that in turn could impair its function.

Table 1

Demographic information of AD and amnesic mild cognitive impairment (MCI) subjects and their respective age-matched controls. The values reported are the average of 6 samples.

A)		
Subjects demographics data	Control (MCI)	MCI
Number of subjects	6	6
Sex	2M, 4F	2M, 4F
Age (years)	82 (74–93)	89 (82–99)
Brain weight (g)	1204 (1080–1315)	1102 (930–1200)
PMI (hours)	1.75–4.00	2.00–5.00
Braak stage	I–II	III–V
B)		
Subjects demographics data	Control (AD)	AD
Number of subjects	6	6
Sex	5M, 1F	2M, 4F
Age (years)	81 (72–87)	85 (80–92)
Brain weight (g)	1219 (1020–1410)	1104 (835–1260)
PMI (hours)	2.00–3.75	2.00–5.75
Braak stage	I–III	V–VI