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Redox Proteomics Identification of Oxidatively Modified Brain Proteins in Alzheimer's Disease and Mild Cognitive Impairment: Insights into the Progression of this Dementing Disorder

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Abstract. Alzheimer disease is a common age-related neurodegenerative disease characterized pathologically by senile plaques, neurofibrillary tangles, synaptic disruption, and progressive neuronal deficits. The senile plaques contain amyloid-β (1–42) and amyloid-β (1–40), that has been shown by a number of laboratories to induce oxidative stress and as well as neurodegeneration, although the exact mechanisms remained to be defined. Our laboratory showed an increased oxidative stress in AD and MCI brain as indexed by protein oxidation and lipid peroxidation. In the present review, we summarize our finding of oxidatively modified proteins using a redox proteomics approach in AD and MCI brain to investigate the mechanism that may be involved in MCI and AD pathogenesis and discuss our findings in terms of AD progression and pathogenesis.

Keywords: Alzheimer's disease, mild cognitive impairment, oxidative stress, amyloid, redox proteomics

INTRODUCTION

Alzheimer's disease (AD) is an age-related neurodegenerative disease that affects approximately 5 million persons in the US population, and in a few decades more than 14 million individuals will be at risk if either the progression of the disease is not slowed or a cure is not identified. AD brain is characterized pathologically by senile plaques (SP), neurofibrillary tangles (NFT), synaptic disruption, mitochondrial oxidative damage and progressive neuronal deficits [10,48,77]. In addition to the formation of SP and NFT, gliosis, chronic inflammatory reactions, excitotoxic damage and oxidative stress all appear to contribute to the progression of AD. The inflammatory processes observed in AD are proposed to involve astrocytes and microglia [99]. Amyloid-β (Aβ) can induce a cascade of cellular mechanisms that includes activation of astrocytes, which leads to neuronal damage [45,49]. In addition, microglia cells are found to be closely associated with neuritic and Aβ plaques [78]. The process of glial activation was characterized by upregulation of molecules such as cytokines that include: interleukin-1β (IL-1β), transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), neurotrophic molecules, various components of complement proteins and reactive oxy-

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gen species, and neurotoxic products [54,125]. Several lines of evidence have shown that the NF-κB in microglia is activated by β-amyloid [30,67]. Further, activation of NF-κB can stimulate increased expression of TNF-α, IL-1, IL-6, NOS etc [1,33], that could eventually lead to increased modification of the proteins. However, in the absence of additional research, the exact role of inflammation in AD pathogenesis is unclear.

Further mutations of the genes for presenilin-1 (PS-1), presenilin-2 (PS-2) and amyloid precursor protein (APP) have been observed in inherited AD. Moreover, there is also a risk association between AD and genes for allele 4 of the apolipoprotein E (APOE) gene, endothelial nitric oxide synthase –3, alpha-2-macroglobulin, and sortilin-related receptor SOLR1 [97]. Other risk factors for AD include aging, stroke, hypertension,diabetes, atherosclerosis, and hypercholesterolemia [25,53,58,79], and combination of these factors further increases the risk of AD [71].

ROLE OF AMYLOID IN OXIDATIVE STRESS

Our laboratory and others have reported that AD brain is under oxidative stress, suggesting an important role for oxidative stress in the pathogenesis and/or progression of AD [14,15,64,69,74,103]. Oxidative stress occurs due to an imbalance in the oxidant and antioxidant levels. Oxidants can damage virtually a plethora of cellular macromolecular targets, including proteins, lipids, carbohydrates, DNA and RNA. In AD brain, the levels of antioxidants were found to be decreased with an associated increase in protein oxidation (protein carbonyl and 3-nitrotyrosine), lipid peroxidation, DNA oxidation, advanced glycation end products, and reactive oxygen species (ROS) formation, among other indices, strongly suggesting a role for oxidative stress in the pathogenesis of AD [14,15, 26–28,64,69,74,104,111,112,114,115]. The highly reactive products of lipid peroxidation include, among others: acrolein, isoketals/neuroketals, 4-oxo-nonenal, 4-hydroxy-2-nonenal (HNE). Among these products HNE and 2-propenal (acrolein) can react with cysteine, histidine, and lysine residues of protein via Michael addition [13,15,23,64,75,129]. Further, in vitro models of AD such as neuronal cells and synaptosomes, treated with Aβ (1–42) were shown to increase the levels of all the oxidative stress parameters including HNE [64,73]. Since HNE is a highly reactive molecule, under physiological conditions HNE is removed as a GSH conjugate by the actions of glutathuione- S-transferase (GST) and the multidrug resistant protein-1 (MRP-1) [88]. A previous study showed that the activity of GST is decreased in AD brain and also in cell culture treated with Abeta [68]. In contrast, Volkel et al., (2006) [122] proposed increased reaction of HNE with GSH or decreased clearance of the HNE-GSH conjugates by peptidases as contributors to increased levels of HNE-GSH rather than alteration in GST activity. Further, a previous study showed an increase in aldehyde dehydrogenase activity, and suggested its role in the mechanisms of HNE detoxification [90]. In addition, our laboratory showed the oxidative modification of both GST and MRP-1 proteins in AD brain, a result that could further provide explanation to the observed increase in protein-bound HNE that has been reported in AD brain [110].

Further, the use of vitamin E in cell culture diminishes Aβ (1–42)-induced toxicity, further consistent with a role of oxidative damage in AD pathology [8,14,128]. A dual role exists between Aβ and the production of free radicals. Aβ peptide can not only induce free radical production under *in vitro* conditions but it can also transform non-aggregated Aβ into aggregated Aβ *in vitro* [43,86]. Mass spectrometry and electron paramagnetic resonance spin trapping (EPR) indicate the Aβ, in aqueous solution, generates free radical peptides [11, 102,119,126]. A number of studies showed impairment of mitochondrial metabolism in AD patients [24, 85,95]. In addition, a number of *in vitro* studies of Aβ and mitochondrial function have reported that Aβ affects mitochondrial protein DNA and proteins, impairments of mitochondrial energy metabolism and ultimately leads to mitochondrial dysfunction [72,92]. Further, an increase in mitochondrial oxidative stress has been reported in AD and its related models; however, the underlying mechanism(s) of these Aβ-induced alterations is (are) still not fully understood [95,127].

Our laboratory has reported that Abeta (1–42) is toxic and the methionine at residue 35 of Aβ (Met35) plays a crucial role in the Abeta-induced oxidative stress [18, 59]. Our hypothesis is supported by studies from various other laboratories [31,34,82]. In one of our studies, we substituted methionine by norleucine. This peptide abolishes free radical production, protein oxidation and toxicity to hippocampal neurons [3,120,127]. We have used various *in vivo* and *in vitro* models to test the role of Aβ in oxidative stress. Others demonstrated that when Aβ (1–40) is subjected to pulse radiolysis, a one-electron oxidation, with formation of sulfuranyl free radical on Met-35 results. The reverse pep-

tide, Aβ (40-1), which is non-neurotoxic, forms a Tyr free radical. This latter is an absolute requirement by those who propose Cu(II) binding and reduction as the source of the oxidative stress and neurotoxic properties of Aβ [56].

In addition, Aβ (1–42) can bind to receptors on neuronal and glial cells, e.g., the α7-nicotinic acetylcholine receptor, neurotrophin p75 receptor, the N-methyl-D-aspartate receptor, the receptor for advanced glycation end products (RAGE) [121,123], and others, forming calcium and potassium channels in cell membranes [5,41], decreasing glucose transport across brain endothelial cells [7], and activating the release of chemokines [42] and cytokines [1]. In the present review we discuss protein oxidation and lipid peroxidation in AD brain.

Aβ exist in various forms, i.e., monomer, oligomers and fibrils. But the form of Aβ that is the toxic species is still largely unknown. Our laboratory used *C. elegans* strains (CL 1175 and CL 4176) as an *in vivo* model to test Aβ associated toxicity. Oxidative stress occurred at 24 h of *in vivo* production of human Aβ(1–42) but no fibrillar Aβ was found [40]. The results of this study are consistent with the notion that the oligomeric form of Aβ is associated with oxidative stress in *in vivo* conditions.

Oxidative stress may cause reversible and/or irreversible modifications on sensitive proteins leading to structural, functional and stability modulations [84, 106]. Protein modifications are generally associated with loss of function and may lead to either the unfolding and degradation of the damaged proteins, or aggregation leading to accumulation as cytoplasmic inclusions, as observed in age-related neurodegenerative disorders [35]. Oxidized proteins are highly sensitive to proteolytic degradation by the proteasome [46, 107]. The increase in the level of oxidized proteins in AD brain is associated with loss of the activity of the 20S proteasome, which represents a major enzyme for the degradation of oxidized proteins [61,89,108, 116]. However, a recent study has questioned these findings [44]. Other studies have shown that prolonged oxidized proteins are more resistant to degradation by the 20S proteasome [96,100].

Previous investigations have used immunoprecipitation techniques to identify specific protein targets of oxidation. This procedure is labor-intensive and time-consuming and requires a good guess to the identity of the protein at the beginning. That is, this approach requires a prior knowledge of the protein so the correct antibody for the protein of interest can be used. We used this approach in initial studies to show that creatine kinase (CK) is oxidatively modified in AD brain [2]. CK was already reported to show a diminished activity in AD brain [50]. Further, posttranslational modification of proteins can sometime alter the structure of proteins [109], which could then prevent the formation of the appropriate antigen-antibody complex. With advances in technology, proteomics coupled to mass spectrometry has been a major methodological development that allows the identification of large number of proteins at once [17]. In our laboratory we have used redox proteomics, that branch of proteomics that identifies oxidatively modified proteins [36], to identify the protein targets of oxidation in AD brain and other neurodegenerative disorders.

REDOX PROTEOMICS

Redox proteomics, which involves the coupling of 2-D gel electrophoresis separation of proteins with mass spectrometric techniques, is a valuable modality to determine oxidatively modified brain proteins [36]. Our laboratory used proteomics to identify a large number of oxidatively modified proteins that were previously undetected by other methods like immunoprecipitation. One of the advantages of proteomics is that total proteome information of the cell, which is constantly changing through its biochemical interactions with the genome and environment, can be obtained in favorable cases. Proteomics can determine protein modifications, localizations, effects of added agents (e.g., drugs) and protein-protein interactions in addition to protein expression levels.

A number of new approches have been added to proteomics methodologies, but still proteomics relies heavily on two-dimensional electrophoresis as the underlying separation technology. This technique combines two important physico-chemical properties, i.e., isoelectric focusing in the first dimension and relative mobility in the second dimension [94]. Figure 1 shows a schematic representation of the proteomics protocol we use in our laboratory. For detailed information regarding the methodology please refer to some of our recent review articles, for example [20,114].

Redox Proteomics Studies Of Alzheimer'S Disease Brain

As noted, AD brain has been shown to have an elevated level of oxidative stress [15]. We previously

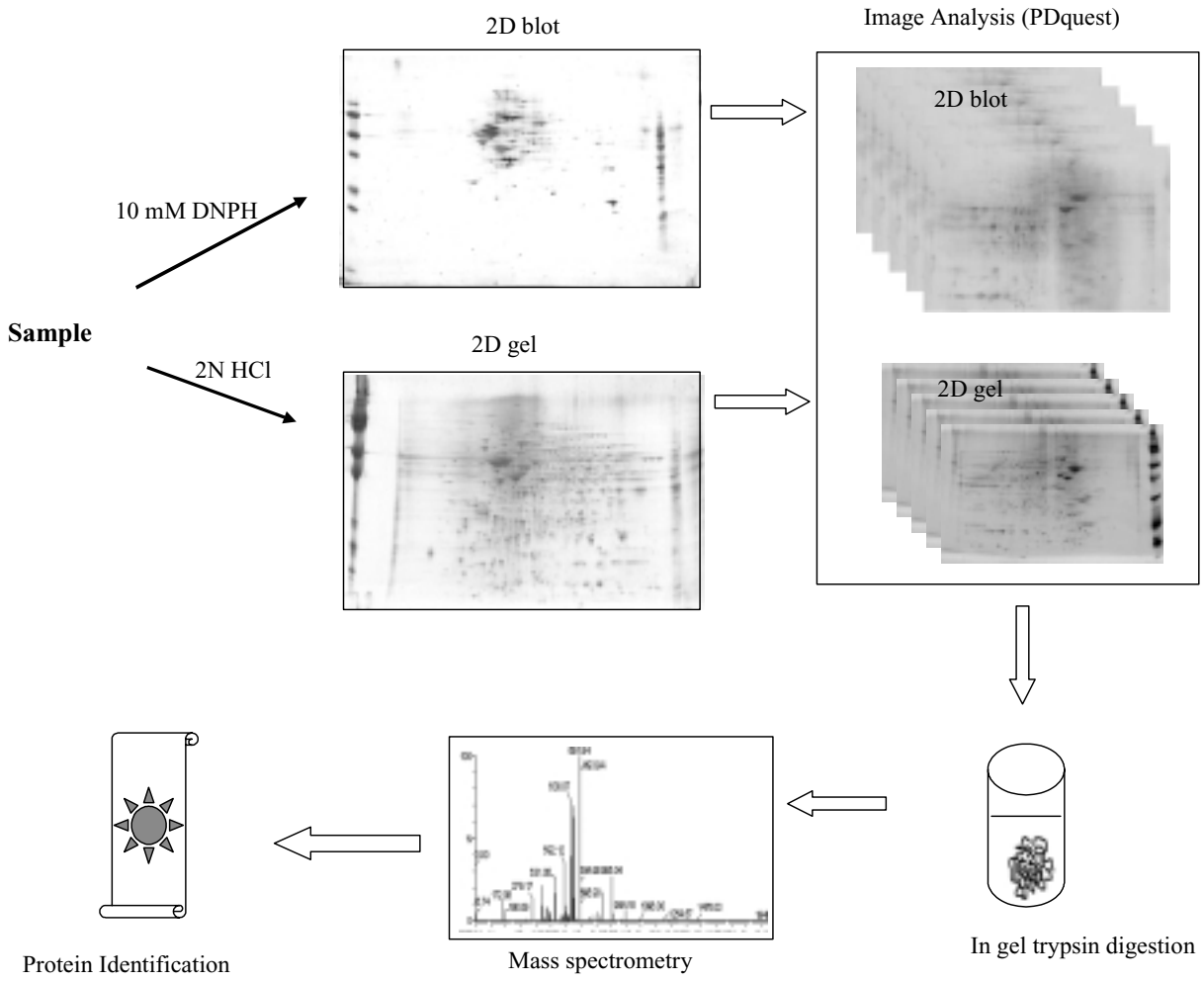

Fig. 1. Redox proteomics protocol used in our laboratory for the identification of oxidatively modified proteins indexed by protein carbonyls.

showed that regions of AD brain rich in Aβ have protein oxidation, while Aβ poor cerebellum does not [50].

Our laboratory was the first to use redox proteomics to identify oxidatively modified proteins in AD brain [9, 16,17,20,26–28,36,111,112,115]. We have identified a number of proteins that are oxidatively modified in AD brain that are listed in Table 1 based on their functions and linked to the observed AD pathology in Table 1. The proteomics identified oxidized proteins include: creatine kinase BB (CK), glutamine synthase (GS), ubiquitin carboxy-terminal hydrolase L-1 (UCH L-1), dihydropyrimidinase related protein 2 (DRP2), α-enolase, phosphoglycerate mutase 1, γ-soluble NSF attachment protein (SNAP), carbonic anhydrase II (CA-II), peptidyl prolyl cis-trans isomerase (Pin 1), neuropolypeptide h3, triosephosphate isomerase and glyceraldehyde 3-phosphate dehydrogenase. The fold-increase in the amount of protein carbonylation and nitration of the reported proteins are shown in Tables 2 and 3, respectively. Further, the identified oxidatively modified proteins were found to be dysfunctional [16, 17,20].

In the present review we have briefly outlined the possible relationship of the identified oxidized proteins in AD with the known pathology of AD.

Energy dysfunction

Creatine kinase (BB isoform), α-enolase, triosephosphate isomerase, glyceraldehyde 3 –phosphate dehydrogenase, phosphoglycerate mutase 1, voltage dependent anion channel protein 1, and alpha ATPase are enzymes involved in energy production. Previous studies using PET showed a reduction of the cerebral metabolic rate of glucose utilization in the AD brain [124]. Our

Table 1
Oxidatively modified proteins identified in AD and MCI brain using redox proteomics. Oxidized proteins found in common between AD and MCI brain are indicated in bold

Functions	AD	MCI
Energy dysfunction	CK, **Enolase**, TPI, PGM1, LDH, GAPDH, ATP synthase alpha, **Enolase** **VDAC**	
Excitotoxicity	EATT2, **GS**	GS
Proteasomal dysfunction	UCH L1, HSC 71	–
Lipid abnormalities and Cholinergic dysfunction	Neuropoly-peptide h3	–
PH buffering and CO_2 transport	CA 2	–
Neuritic abnormalities	DRP2, β-actin	–
Tau hyperophosphoryaltion/ Aβ production/ mitosis	**Pin 1**	**Pin 1**
Synaptic abnormalities and LTP	γ-SNAP	–
Pyruvate Kinase M2	–	Pyruvate Kinase M2

AD = Alzheimer's disease, MCI = Mild cognitive impairment, CK = Creatine kinase BB, TPI = Triose phosphate isomerase, PGM1 = Phosphoglycerate mutase 1, LDH = Lactate dehydrogenase, EATT2 = excitatory amino acid transporter 2, GS = Glutamine synthase, UCHL1 = ubiquitin carboxy-terminal hydrolase L-1, HSC 71 = Heat shock cognate 71, DRP2 = dihydropyrimidinase-related protein 2, Pin1 = peptidyl-prolyl cis-trans isomerase, γ-SNAP = gamma-Soluble NSF-attachment proteins, VDAC = Voltage dependent anion channel protein.

findings that proteins involved in glucose metabolism are oxidized proteins suggest disturbances in memory formation and memory retrieval [51], as is found for example in sporadic AD [52]. In addition to oxidation, the functions of all these proteins except triosephosphate isomerase in AD were found to be altered which could severely affect the total ATP production in the cell. Such alterations in ATP production could lead to cellular dysfunction such as impaired ion-motive ATPase activity to maintain potential gradients, operate pumps, and maintain membrane lipids asymmetry, etc. Loss of membrane lipid asymmetry induced by Aβ or HNE has been reported previously from our laboratory [29,80]. Also ATP diminution can induce hypothermia that could lead to abnormal tau hyperphosphorylation through differential inhibition of kinase and phosphatase activities, ion pumps, electrochemical gradients, cell potential, voltage-gated ion channels [91].

Excitotoxicity

Glutamate synthase (GS) and excitatory amino acid transporter 2 (EAAT2) are involved in glutamate reuptake or conversion. Glutamine synthase converts glutamate and ammonia into glutamine. In AD brain glutamine synthase enzymatic activity has been shown to be decreased [12,50,65]. Interestingly, reduced GS activity was localized to brain areas with increased oxidation products mostly present in the vicinity of Aβ plaques [101]. Further, our laboratory also identified EAAT2 as an HNE-bound protein in AD by immunochemical methods, and we replicated the oxidation of the EAAT2 in AD brain using a synaptosomal model subjected to Aβ (1–42)-induced oxidative stress [64]. HNE, a product of lipid peroxidation has been shown to induce conformational changes in proteins [109] that could eventually impair the functionality of proteins. Our findings of the oxidation of these proteins are consistent with increased excitotoxicity and neurodegeneration that support the possibility that abnormal functioning of this system might be involved in the pathogenesis of synaptic damage in AD.

Proteasomal Dysfunction

Ubiquitin carboxyl-terminal hydrolase L-1 (UCH L-1) is an enzyme that that removes ubiquitin from the protein-bound poly (ubiquitin) chain prior to insertion of the damaged protein into the core of the 26S proteasome, i.e., a signal for protein degradation. UCH L-1, helps to maintain the pool of ubiquitin in cells. UCH L-1 was identified as one of the oxidized proteins in AD brain [26,112], and the oxidation of this protein would decrease the activity or lead to inactivation of protein functions [29], which could lead to depletion of the free pool of ubiquitin or cause saturation of the proteasome with polyubiquitin chains [93]. Oxidation of UCH L-1 could also lead to oxidative stress [38] in addition to the accumulation of the damaged proteins, synaptic deterioration and neurodegeneration in AD. Proteomics identification of UCH L-1 as an oxidized protein suggests a role of oxidative stress in proteaso-

Table 2
Fold-increase of carbonylated proteins in AD IPL, AD Hippocampus, and MCI hippocampus relative to respective control brain regions

Carbonylated proteins	Fold-increase in Oxidation	References
AD-Inferior Parietal Lobule		
DRP-2	4.89 ± 0.52	27
$\alpha-$enolase	3.21 ± 0.18	27
Heat shock cognate-71	2.24 ± 0.63	27
Creatine kinase BB	4.89 ± 0.52	26
Glutamine synthase	3.21 ± 0.18	26
Ubiquitin carboxyl-terminal hydrolase L-1	2.24 ± 0.63	26
AD-Hippocampus		
Peptidyl prolyl *cis/trans* isomerase 1	1.36 ± 0.55	112, 111
DRP-2	1.26 ± 0.45	112
Phosphoglycerate Mutase 1	212.30 ± 266.8	112
Carbonic anhydrase	3.27 ± 0.85	112
Enolase 1	2.55 ± 0.62	112
Triose phosphate isomerase	6.44 ± 2.28	112
Gamma-SNAP	3.15 ± 132	112
UCHL-1	2.10 ± 0.45	112
MCI-Hippocampus		
Enolase 1	3.5	21
Glutamine synthetase	4.0	21
Pyruvate kinase M2	3.0	21
Peptidyl prolyl cis/trans isomerase	5.0	21

mal dysfunction and AD [26,112]. In addition, UCH L-1 rescues Aβ-induced decreased synapatic function and contextual memory [104], suggesting that oxidatively dysfunctional UCH L-1 could contribute to the known memory defects in AD.

Lipid Abnormalities And Cholinergic Dysfunction

In AD brain neuropolypeptide h3, a phosphatidylethanolamine binding protein [PEBP] or hippocampal cholinergic neurostimulating peptide [HNCP], has been identified as a specifically oxidized protein [28]. PEBP plays an important role in maintaining phospholipid asymmetry, which is important to maintain the structure and function of membranes [29,81]. The oxidation of this protein could lead to the loss of PEBP activity that may lead to loss of membrane asymmetry, which, in turn, may initiate apoptosis and consequently to cell death. Our laboratory showed that the addition of Aβ (1–42) and HNE to synaptosomes lead to loss of phospholipid asymmetry [81]. This enzyme also regulates the levels of choline acetyltransferase, an enzyme that is reported to have decreased activity in AD brain [63], and this could be related to the reported cognitive decline in AD.

Neuritic Abnormalities

Dihydropyrimidinase related protein 2 (DRP-2), and β-actin are structural proteins that are found to be oxidized in AD brain [27,112]. DRP2 is normally expressed in developing brain and found only sparingly in adult brain. The oxidation of actin could be related to the loss of cytoskeletal network integrity and activation of cellular events that may lead to apoptosis. The oxidation of DRP-2 could impair interneuronal communication and repair and also interfere with the regulation of the activity of collapsin, a protein that is involved in dendritic elongation and pathfinding [47,60]. In AD brain, oxidation of these proteins could be related to the observed shortened dendritic length [32] and cognitive impairment in AD [57].

Tau Hyperphosphorylation/Aβ Production/Prevention Of Exit Of Neurons From Mitosis

Peptidyl-prolyl cis/trans isomerase (Pin 1) was found to be one of the oxidatively modified proteins in AD hippocampus with an associated decrease in enzyme activity [111,112]. This protein binds to a phosphorylated serine or threonine on the N-terminal side of a proline of target proteins. Pin 1 catalyzes the conversion of the *cis* to *trans* conformation and vice versa of the proline in target proteins, thereby conformationally regulating target protein activity. Pin 1 regulates activity of protein phosphatase 2A (PP2A), which dephosphorylates tau, and GSK-3β, which phosphorylates tau. Recent studies show that Pin1 is colocalized with phosphorylated tau and also shows an in inverse relationship between expression of tau and Pin 1 in Alzheimer's tautopathies [70]. Pin 1 also modulates Aβ production by regulating APP, thereby keeping the Aβ levels low [87]. Pin 1 protein also prevents neu-

Table 3
Fold-increase of nitrated proteins in AD IPL and Hippocampus relative to respective control brain regions

Nitrated proteins	Fold-increase in Oxidation	References
AD-Inferior Parietal Lobule		
$\alpha-$Enolase	3.12 ± 0.87	28
Triosephosphate isomerase	4.8 ± 2.09	28
Neuropolypeptide h3	7.65 ± 3.71	28
$\beta-$Actin	1.44 ± 0.68	28
l-Lactate dehydrogenase	1.62 ± 1.18	28
$\gamma-$Enolase	1.53 ± 1.11	28
AD-Hippocampus		
Alpha- Enolase	3.47 ± 0.90	114
Carbonic anhydrase II	2.53 ± 0.72	114
Glyceraldehyde-3-phosphate dehydrogenase	2.18 ± 0.64	114
ATP synthase alpha chain	3.26 ± 1.70	114
Voltage-dependent anion-channel protein-1	5.11 ± 1.20	114

ronal cells from exiting mitosis. Therefore, oxidatively dysfunctional Pin 1 may be critically important in the known major pathologies of AD, i.e., hyperphosphorylation of tau (NFT), increased production of Aβ (SP), and loss of neurons or synapses due to cell cycle machinery failure [19,70,87,105].

Synaptic Abnormalities And LTP

γ-SNAP, a member of synaptosomal protein-like soluble N-ethylmaleimidesensitive factor (NSF) attachment proteins (SNAPs) play an important role in vesicular transport in the constitutive secretory pathway as well as in neurotransmitter release, hormone secretion and mitochondrial organization. Our group identified this protein as an oxidatively modified protein in AD hippocampus [112], which could be related to the reported loss of synaptic circuitry in AD brain, alterations in neurotransmitter system and cognitive deficits in AD individuals.

pH Buffering And CO_2 Transport

Carbonic anhydrase II is identified as an oxidatively modified protein in AD hippocampus and is also reported to have decrease activity [112,115]. Since CA 2 plays an important role of regulating the pH of cell, which is crucial for maintaining the activity of enzymes, mitochondrial pH-dependent ATP production, and other pH sensitive aspects of neurons could be jeopardized. In addition CA 2 also regulates the production of CSF, and the synthesis of glucose and lipids. As noted, CA 2 oxidation in AD brain may lead to an imbalance in both the extracellular and intracellular pH in the cell, mitochondrial alterations in oxidative phosphorylation, and impaired synthesis of glucose and lipids.

Redox Proteomics In MCI Brain

Both in MCI and AD patients, plasma mean levels of non-enzymatic antioxidants and activity of antioxidant enzymes are lower compared to controls, and there is no parallel induction of antioxidant enzymes [62]. These data suggest that increased free radical production in MCI might lead to a rapid consumption of plasma antioxidants without a simultaneous activation of new molecules of antioxidant enzymes. MCI and subsequently AD individuals are likely to have an inadequate antioxidant enzymatic activity that might account for increased free radical production and may be one of the mechanisms of AD pathogenesis. Recently our group showed that subjects with MCI have increased protein oxidation and lipid peroxidation in hippocampus and IPL [21,22,62]. Others observed different markers of oxidative stress in MCI brain [21,22,62,76]. This suggests that the increased levels of protein and lipid peroxidation could be early events in AD pathophysiology. Since MCI is the considered as a transition stage between control and early stage of AD, the finding of increase oxidative stress suggests that oxidative stress is an early event in the progression of AD [21,22].

In order to better understand the role of oxidative stress in AD pathology, our laboratory also studied MCI brain samples by redox proteomics to identify any commonly oxidized proteins in AD and MCI. There were three proteins oxidatively modified in common between MCI and AD: enolase; Pin1; and GS [21]. Further, these proteins that were identified to be oxidatively modified were found to be dysfunctional in MCI and AD [21]. The identification of Pin1 to be oxidatively modified may further account for the elevated levels of cell cycle proteins in MCI brain [113]. The data obtained from our laboratory suggest that the oxidation of these key proteins are important in the conversion

of MCI to AD [21]. These oxidatively dysfunctional proteins in arguably the earliest form of AD may be important in the conversion from MCI to AD and not simply a consequence of the disease. In addition, mutations in APOE, presenilin 1 and the amyloid precursor protein [83,118] have been also observed in subjects with MCI.

Oxidative stress and redox proteomics identification of oxidized proteins in AD and MCI provide new insights into potential mechanisms of neurodegeneration in these disorders. Indeed, each of the proteomics identified proteins whose activities are altered can be invoked in the plausible mechanisms of neurodegeneration. Further, the modified proteins potentially could be markers of disease. There is a large body of evidence implicating both mitochondrial dysfunction and oxidative damage in the pathogenesis of AD. Hence, enhancement of antioxidant levels in mitochondria may be important in AD treatment. A prior study showed that vitamin E has efficacy in slowing the progression of AD [98,117]. Ginkgo biloba also may exert beneficial effects [6,37,39]. The antioxidants curcurmin and melatonin exert beneficial effects on amyloid deposition in transgenic mouse models of AD [55,66,72]. Recent studies have identified MitoQ, MitoVitE, MitoPBN, MitoPeroxidase, and amino acid and peptide-based SS tetrapeptides as good antioxidants that can rapidly neutralize free radical and mitochondrial toxicity [95]. In addition, recently from our laboratory we showed that D609, a glutathione mimetic, protects brain mitochondria against Aβ-induced toxicity [4].

Our findings presented in this review are consistent with the Aβ-associated free radical oxidative stress model of neurodegeneration in AD brain [14,120] and imply that AD may benefit from therapeutic intervention by appropriate, brain accessible endogenous and exogenous antioxidants.

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