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Adventiously-bound redox active iron and copper are at the center of oxidative damage in Alzheimer disease

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Abstract

Central to oxidative damage in Alzheimer disease is the production of metal-catalyzed hydroxyl radicals that damage every category of macromolecule. Studies on redox-competent copper and iron indicate that redox activity in Alzheimer disease resides exclusively within the cytosol of vulnerable neurons and that chelation with deferoxamine or DTPA removes this activity. We have also found that while proteins that accumulate in Alzheimer disease such as tau, amyloid beta, and apolipoprotein E possess metal-binding sites, metal-associated cellular redox activity is more dependent on metal-nucleic acid binding. Consistent with this finding is the large amount of cytoplasmic RNA in pyramidal neurons. Still, the source of metal-catalyzed redox activity is controversial. Heme oxygenase-1, an enzyme that catalyzes the conversion of heme to iron and biliverdin, is increased in Alzheimer disease suggesting increased heme turnover as a source of redox-active iron. Additionally, the role of mitochondria as a potential source of redox-active metals and oxygen radical production is assuming more prominence. In recent studies, we have found that while mitochondrial DNA and cytochrome C oxidase activity are increased in Alzheimer disease, the number of mitochondria is decreased, indicating accelerated mitochondria turnover. This finding, as well as preliminary studies demonstrating a reduction in microtubule density in neurons in Alzheimer disease suggests mitochondrial dysfunction as a potentially inseparable component of the initiation and progression of Alzheimer disease.

Introduction

Oxidative damage to every category of biomacromolecule – sugars, lipids, proteins and nucleic acids – is increased in Alzheimer disease (AD) (Perry *et al.* 1998). Early studies of oxidative damage in AD had suggested that the increase involved chemical alterations to lesions (Ledesma *et al.* 1994; Mattson *et al.* 1995; Smith *et al.* 1995), since they had long residence times allowing them time to accumulate modifications similar to that found in vascular basement membrane (Sayre *et al.* 1999). However, more recent studies have shown that the major site at which these modifications occur is the neuronal cytosol, prior to the formation of any lesions (Smith *et al.* 1996, 1997; Sayre *et al.* 1997). This article explores what a cytosolic site of damage and the broad range of changes can tell us about the mechanisms responsible for damage, focusing on the role of redox-active metals.

The various types of damage noted in AD (Table 1) are well established forms of modification, which result directly or indirectly from metal-catalyzed •OH production. One exception is tyrosine nitration, which has been considered to result exclusively from peroxynitrite, a product of O_2^- and NO and not requiring metals (Beckman *et al.* 1994). However, several recent studies have challenged the exclusivity of nitrotyro-

Table 1. Oxidative modification found in Alzheimer disease.

Macromolecule	Modification
Sugars	Glycation
Protein	Carbonyls, nitration
Lipids	Hydroxynonenal, acrolein
Nucleic acid	8-hydroxyguanosine

sine addition in peroxynitrite by demonstrating that myeloperoxidase can catalyze tyrosine nitration using NO_2 and H_2O_2 as substrates (Sampson *et al.*, 1998). In preliminary experiments, we have found that de-novo-nitration of tyrosine with H_2O_2 and nitrate can be accomplished by bound metals and is restricted to the same vulnerable neurons noted *in vivo*. In contrast, treatment with peroxynitrite lacks similar specificity. In sum, metals play a central role in oxidative damage in AD.

To explore the cellular location of redox active copper and iron, we used the ability of redox-competent metals to catalyze the oxidation of a substrate in the presence of H_2O_2. After application of H_2O_2 to tissue sections with the oxidizeable substrate diaminobenzidine, sites of redox activity are readily apparent and reside exclusively within the neuronal cytoplasm (Sayre *et al.* 2000). Treatment with either deferoxamine or chelation by DTPA blocks this activity, while subsequent reapplication of either copper or iron restores activity. This clearly demonstrates that cellular redox activity is completely dependent on exchangeable metals. While τ (Sayre *et al.* 2000), amyloid β (Cuajungco *et al.* 2000) and apolipoprotein E (Miyata & Smith 1996) have all been shown to possess metal binding sites, metal-associated cellular redox activity depends to a greater extent on metals associated with nucleic acid since pretreatment with RNase A or DNase I significantly reduces activity. There was also a complete reduction by S1nuclease, an enzyme with specificity for non-base-paired RNA or DNA nucleotides. Since RNA has far more non-base paired regions than DNA and oxidative damage to nucleic acids in AD is more extensive in RNA than DNA (Nunomura *et al.* 1999), it seems likely that metals bound to RNA are the major sites of redox activity, particularly since •OH reacts with the first molecule it encounters. The importance of proteins to these binding sites remains to be determined, but we now know that metal-catalyzed redox activity, whether it be cytoplasmic, or in senile plaques and neurofibrillary tangles, is dependent on nucleic acid metal binding sites.

Since neurons contain abundant cytoplasmic RNA, predominantly in the form of ribosomal RNA (rRNA), it is no surprise that when iron is added to sections from control cases, metal-catalyzed redox activity is generated, while little activity is seen in the absence of added metal. Therefore, while the metal binding sites may be increased in AD compared to controls, what is clearly important is the increase in the metals themselves. This finding led us to consider possible sources of increased metals. Several years ago, the enzyme heme oxygenase-1 (HO-1) was found to be induced in AD (Smith *et al.* 1994; Premkumar *et al.* 1995; Schipper *et al.* 1995). HO-1 catalyzes the conversion of heme to iron and biliverdin, which is subsequently reduced to the antioxidant bilirubin. HO-1 is induced by increased heme levels (Keyse & Tyrrell 1989) suggesting an abnormality in heme turnover might be associated with AD.

Since many heme-containing enzymes are accumulated in mitochondria, we examined whether there might be mitochondrial abnormalities in AD. Our approach was to examine mtDNA at the cellular level, mitochondrial proteins, mitochondrial enzyme activity, and mitochondrial structure in AD and controls. We found a 3–4-fold increase in the mitochondrial protein, COX-1 and mtDNA specifically in vulnerable neurons (Hirai *et al.* 2001), yet paradoxically, we also found, as did prior studies (Wong-Riley *et al.* 1997), that there was no increase in mitochondrial enzyme activity (Figure 1). Morphometric ultrastructural analysis of biopsy specimens indicated that, if anything, mitochondria are reduced in AD (Hirai *et al.* 2001). Ultrastructural *in situ* hybridization and immunocytochemistry showed that the increased mtDNA and proteins, instead of being mitochondria were in autophagosomes, i.e., not in intact mitochondria, but rather mitochondria being turned over. Ultrastructural localization of redox activity showed that autophagosomes, particularly the residual body of lipofuscin, contained abundant activity in addition to ribosomes consistent with metals bound to rRNA. These findings suggest that mitochondrial enzyme turnover in lysosomes is the likely source of the increased heme turnover, which in turn results in HO-1 induction.

Further morphometric analyses of biopsy specimens suggests why mitochondria in AD are targeted to lysosomes. The number of neuronal microtubules in AD is reduced by half, irrespective of whether the

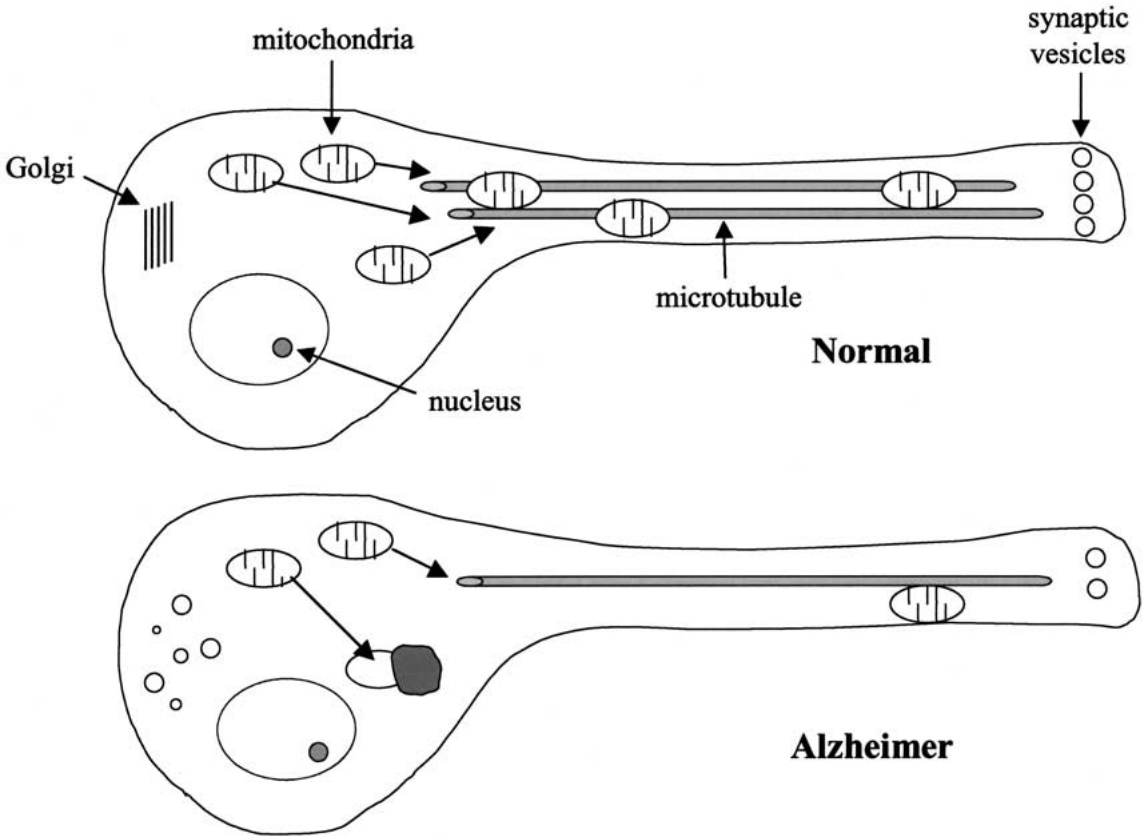

Fig. 1. Hypothesis: Reduced microtubules and the transport processes dependent on them lead to Golgi fragmentation, retention of mitochondria in the cell body with targeting to autophagosomes and reduced synaptic vesicles at terminals.

neurons contain neurofibrillary tangles. Since mitochondria generated in the cell body are transported through the axon on microtubules, a decrease in microtubule number would leave the mitochondria to either accumulate or be turned over in the cell body. Our observations suggest that mitochondrial turnover is responsible for the conditions that promote oxidative damage in AD. Changes in microtubules have been suggested for over 30 years to underlie AD (Suzuki & Terry 1967; Terry 1996, 2000; Terry & Katzman 2001). The observations of diminished synaptic vesicles (Praprotnik *et al.* 1996) and Golgi disruption (Stieber *et al.* 1996) are consistent with the importance of microtubules. Particularly germane to AD is that increased turnover of metalloproteins in the cell body increases the metal burden of this compartment, which, if anything, reduces flux to other compartments. Therefore, metalloprotein turnover and RNA localization are potentially sufficient alone to provide cell body specificity for redox activity and oxidative damage (Figure 2).

This presentation paints a picture of disregulation based on inappropriate membrane trafficking and transport and only requires further definition of metal binding sites for full mechanistic appreciation. Alternatively, we suggest that these changes may be part of an appropriate adaptive response to altered trafficking. We base this hypothesis on our findings of oxidative damage to cytoskeletal proteins in AD. While oxidative modifications to proteins would appear indiscriminate, being controlled only by proximity to reagents, and the nature of the amino acid side chain and half-life, in practice modifications are quite specific to individual proteins. For example, when we examined the human brain for proteins modified by hydroxynonenal (HNE), a reactive aldehyde derived from lipid peroxidation, the vast majority of adduction occurs on neurofilaments (Figure 3). Further, rather than being randomly modified, neurofilaments are modified exclusively on lysine amino groups and this modification is controlled by phosphorylation (Wataya *et al.* 2002). *In vitro*, neurofilament heavy subunit

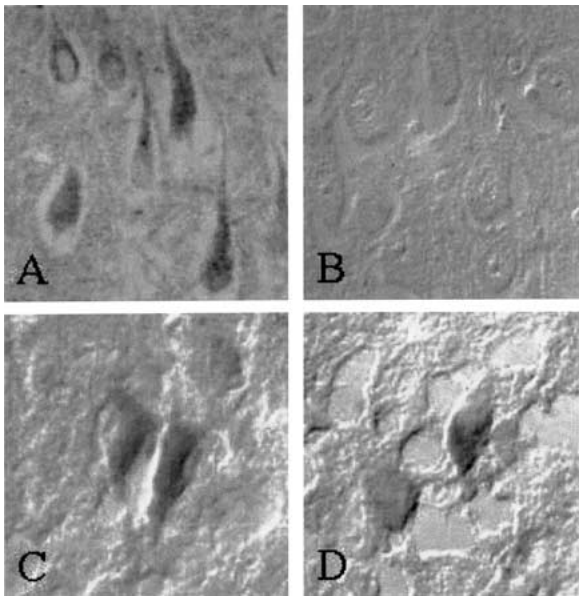

Fig. 2. Cytochrome oxidase protein as detected with a monoclonal antibody (1D6) is present at higher levels in cases of AD (A) as compared to age-matched controls (B). However, cytochrome oxidase activity is found at similar levels in AD cases (C) and controls (D).

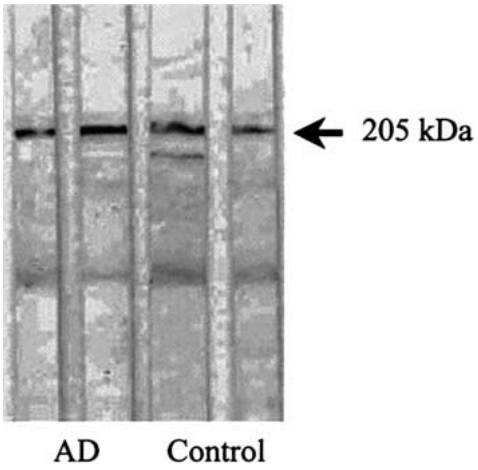

Fig. 3. Immunoblots of AD and control homogenates showing HNE-Michael adducts on NFH protein (250 kDa). Note both AD and controls show HNE adducts, with AD specimens having a higher level of adduction. 10 μg of cortex homogenate in Tris-buffered saline, pH 7.6, was loaded per lane.

(NFH) is highly reactive with HNE, but following dephosphorylation, HNE reactivity is greatly reduced (Wataya *et al.* 2002).

Ribosomes are known to be associated with microtubules in neuronal dendrites. The damage relating in impaired membrane trafficking and transport may then result from oxidative damage to microtubules mediated by the redox active metals associated with rRNA. This is further supported by the observation that tau mRNA co-localizes with tau protein in intact cells (Aronov *et al.* 2001) again juxtaposing the ribosome with the microtubule. Since tau protein synthesis is likely to occur near the growing end of the microtubule, this may explain the loss of microtubules in the AD brain. These observations provide a link between localization of redox active metals and the transport deficits seen in AD.

In review, we present a hypothetical model as the basis of oxidative damage in AD. This model is based on alterations in metalloprotein turnover, particularly from mitochondria and metal binding sites in RNA. The model further suggests that therapeutic efforts to reduce oxidative damage can be approached by improving microtubule transport as well as by metal chelation but may benefit the system best by the former.

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