

Sources and mechanisms of cytoplasmic oxidative damage in Alzheimer's disease

Michael Marlatt, Hyoung-gon Lee, George Perry, Mark A. Smith and Xiongwei Zhu

Institute of Pathology, Case Western Reserve University, 2085 Adelbert Road, Cleveland, Ohio 44106, USA

Review

Abstract. While evidence supports a pathogenic and proximal role for oxidative stress in Alzheimer's disease, the causes and consequences of reactive oxygen species that promote oxidative damage have not been directly demonstrated. Co-incident with the reduced energy metabolism during the development of the disease, some of the key mitochondrial enzymes have shown deficient activity in AD neurons, which may lead to increased ROS production. However, we found that oxidative damage occurs primarily within the cytoplasm rather than in mitochondria. Given that SOD activity is increased in AD mitochondria and that metal ions such as iron and copper are enriched in susceptible neurons, we hypothesize that mitochondria, as a source, provide hydrogen peroxide, which, as an intermediate, once in the cytoplasm, will be converted into highly reactive hydroxyl radicals through Fenton reaction in the presence of metal ion and cause damage in cytoplasm.

The correspondence should be addressed to X. Zhu, Email: Xiongwei.Zhu@cwru.edu

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INTRODUCTION

In recent years considerable advances have been made in the study of Alzheimer's disease (AD). Many of these studies have focused on the oxidative stress and associated factors that concern neurodegenerative disease. Oxidative stress has been implicated with neurodegeneration and modification to essentially every class of biomacromolecules in association with susceptible neurons of AD reflecting oxidative damage (Castellani et al. 1998, Nunomura et al. 2001, Sayre et al. 1997, Xu and Sayre 1998). More recent studies have isolated mitochondria (Hirai et al. 2001) as the possible cause of this stress but surprisingly research findings have also shown that oxidative stress is strictly localized in the cytoplasm of the susceptible neuron (Nunomura et al. 1999, Smith et al. 1997b). Our goal is to provide a possible explanation to this localization paradox.

MITOCHONDRIA ARE A SOURCE OF OXIDATIVE STRESS

All aerobic organisms produce free radicals, predominantly superoxide formed as a byproduct of electron transport in the mitochondria during the reduction of molecular oxygen. It has been estimated that 1% of respired molecular oxygen will form O_2^- . The average cell utilizes 10^{13} O_2 per day thus approximately 10^{11} superoxide radicals are produced by each cell in a day. The production of radicals is heightened in the brain due to the high oxygen metabolism of neurons. Although the brain only constitutes 2-3% of total body mass, it utilizes 20% of basal oxygen supplied to the body. While most of these radicals are sequestered in the mitochondria, oxidative insult is exacerbated by age, metabolic demand, and disease (Smith et al. 1992). The prevalence of neurodegenerative diseases increases with age and supports the idea that oxidative stress is a main factor in neurodegeneration (Gracy et al. 1999).

Mitochondria have been implicated in the earliest stages of Alzheimer's disease through a number of studies (Cash et al. 2002). These studies have focused on the reduced energy metabolism that precedes development of the disease (Blass 2000, Blass and Gibson 1999). Studies utilizing PET scanning of subjects with a heighten ApoE status and have been able to examine the clinical and neuroanatomic features that characterize AD (Reiman et al. 1996). Given these findings it is not surprising that alterations to the mitochondrial enzymes have been found to underlie this deficit in energy metab-

olism. Measurements of the mitochondria enzymes α -ketoglutarate dehydrogenase complex (KGDHC), pyruvate dehydrogenase complex (PDHC), and cytochrome oxidase, have shown deficient activity in AD neurons (Mastrogiacomo et al. 1993, Simonian and Hyman 1994, Yates et al. 1990). The reduced activities of these enzymes indicate dysfunction of the mitochondria in AD that favor the increased production of superoxide. Additional research has shown that mitochondria are reduced in neurons vulnerable to AD and that products of their degradation are increased (Hirai et al. 2001). Studies examining the presence of mitochondrial DNA in AD neurons have further shown that mitochondria are degraded in AD (Hirai et al. 2001), showing consistent correlation with findings that AD neurons have a reduced rate of energy metabolism (Blass 2000).

Although there is no doubt that mitochondria function is compromised in AD favoring the increased production of superoxide, it is interesting that virtually no overt oxidative modification to mitochondria components has been observed (Hirai et al. 2001). This may be due to the enhanced compensation mechanisms in the organelle. The principal metalloenzymes responsible for cellular regulation of reactive oxygen are the Mn and CuZn superoxide dismutases (SOD2 and SOD1, respectively) that remove superoxide by converting it to hydrogen peroxide. Although still controversial, several studies showed that SOD activity is elevated in susceptible neurons in AD (Lovell et al. 1995). More importantly, SOD1 activity is consistently increased by 50% in patients with Down's syndrome (Margaglione et al. 1995), a disease whose clinical symptoms resemble the clinical symptoms of AD. Therefore, increased superoxide production coupled with elevated SOD activity is easily translated into elevated levels of hydrogen peroxide. Removal of ROS such as hydrogen peroxide in mitochondria requires chemical removal of electrons provided by a functional Krebs tricarboxylic acid cycle. However, deficiencies in PDHC and KGDHC in AD, which has adverse effect on the cycle, prevent the completion of this task in mitochondria (Gibson et al. 1998, 2000). Therefore, elevated levels of hydrogen peroxide, which have an ability to pass biological membrane, lead to a greatly increased threat in the surrounding cytoplasm.

HYDROGEN PEROXIDE AS AN INTERMEDIATE

Superoxide dismutase (SOD) is considered the primary defense against buildup of reactive oxygen be-

cause it removes O_2^- , the initial form of metabolically produced reactive oxygen. The product of SOD2 action and monoamine oxidase is hydrogen peroxide (H_2O_2), which is freely diffusible across mitochondrial membrane and can tremendously add to this oxygen radical burden in compartments having less protection from reactive oxygen than mitochondria. Catalase is found exclusively in the cytoplasm of the cell and acts upon H_2O_2 to create water and thus acts as a second step in eliminating superoxide molecules that originally contact SOD enzymes. A limited number of studies have occurred concerning catalase and it remains an area of debate regarding the activity of catalase in the AD neuron (Sagara et al. 1996). However, the most important parameter is the SOD/CAT ratio in susceptible neurons and in fact at least one study clearly demonstrates that SOD/CAT ration increases in AD neurons (Gsell et al. 1995) which suggests that hydrogen peroxide concentration in the cytoplasm is increased. Indeed, several groups have found that AD patients and APP transgenic mice (Takahashi et al. 2000) have increased hydrogen peroxide levels in cytoplasm. Hydrogen peroxide is not a highly reactive molecule however it can react with metals to produce highly reactive hydroxyl radicals that pose a great threat to the brain, thus seemingly harmless accumulations of hydrogen peroxide can become highly detrimental to the neuron.

PRO-OXIDATIVE METAL AS A CATALYST

Hydrogen peroxide will form hydroxyl radicals, the most highly reactive molecules of all categories of biomolecules. Most hydroxyl radicals arise as a consequence of Fenton chemistry between reduced transition metals (usually Fe^{2+} or Cu^{1+}) and H_2O_2 . Therefore increases in H_2O_2 in the presence of metals are associated with increases in highly reactive hydroxyl radicals and increased damage to the cell. The situation is further exacerbated by the facts that the transition metals are dysregulated (Pogocki 2003, Smith et al. 1998) and redox-active transition metals are aberrantly accumulated in cytoplasm in susceptible neurons in AD (Smith et al. 1997a) which may account for the obvious severe oxidative modification to cytoplasmic components (Nunomura et al. 1999).

The effects of increased hydrogen peroxide in the presence of metals can be examined by pursuing the products of these reactions. In a chronic disease like AD

it is important to distinguish rapidly-formed and long-lasting oxidative modifications because the former represents the current oxidative state and the latter represents the history. It is possible to identify the first products of oxidative stress by investigating advanced glycation endproducts (AGE) found in the cell. These molecules result from metal-catalyzed redox chemistry and are continuing sites of redox chemistry (Smith et al. 1991), they are therefore considered "active modifications". Additionally, peroxynitrite, created by the chemical interaction of superoxide and nitrous oxide (NO), will generate oxidative species that oxidize and nitrate proteins (Beckman 1996). Nitration of tyrosine residues has been extensively studied (Greenacre and Ischiropoulos 2001) and has been associated with AD through a number of recent investigations (Castegna et al. 2003). In investigating the distribution of nitrotyrosine with the AD neuron it was found that few neurons with Neurofibrillary tangles (NFT) showed nitrotyrosine (Smith et al. 1997b). Instead, the major site of nitrotyrosine was in the neuronal cytoplasm. Most neurons containing NFT showed lower levels of nitrotyrosine than the surrounding neurons lacking NFT (Smith et al. 1997b). Nitrotyrosine can be formed from peroxidative nitration by nitrite and H_2O_2 (Sampson et al. 1998). Peroxynitrite can react similarly to reactive hydroxyl radicals creating 8OHG and other oxidation products. In the case of peroxidative nitration, treating tissue sections from cases of AD with nitrite and H_2O_2 yields increased nitrotyrosine of the same distribution found during the disease (Perry and Smith, unpublished observation). This observation suggests that nitrotyrosine observed in the AD cases may result from increased peroxide presence. These surprising relationships were additionally seen when a cellular component with higher turnover were examined. A major oxidation product of RNA, 8-hydroxyguanosine (8OHG), has a distribution similar to nitrotyrosine, except that it is absent from neurons containing NFT and reduced in the surrounding cytoplasm (Nunomura et al. 2001). These findings localize the oxidative stress found in AD to the cytoplasm and show that increases in hydrogen peroxide could be related to this stress.

The fact that redox-active metals, primarily iron and copper, are accumulated in the cytoplasm as well as enriched in pathological markers of AD suggests a disturbed homeostasis, which may be due to the dysregulation of cellular metal metabolism. Indeed, an increase in iron concentration with a concurrent de-

crease in ferritin, which would leave the neurons vulnerable to ROS since there is apparently not enough ferritin to detoxify iron, is seen in AD brain (Connor et al. 1995). IRP proteins show significant alterations in AD (Smith et al. 1998), paralleling alterations in redox-active iron (Smith et al. 1997a). Ceruloplasmin, a copper-binding protein that mediates the entry of copper into brain and also plays a role in protecting cells against oxidative damage, is increased in brain tissue and cerebrospinal fluid paralleling the increase of copper in brain (Castellani et al. 1999). However, neuronal levels of ceruloplasmin are not changed which may leave them more vulnerable to the toxic oxidative effect of increased copper.

Other studies have focused on the toxicity associated with the structures found in AD. These studies suggest that conditions known to alter neuronal GSH homeostasis such as amyloid- β in AD (Martins et al. 1986, Pogocki 2003, Russell et al. 1999), are likely to manifest in cellular toxicity due to endogenous copper. Other studies have replicated this effect by artificially controlling the regulation of metals in the cytoplasm (Cherny et

al. 2001, Gnjec et al. 2002, Gouras and Beal 2001). A β toxicity for cells in culture can be potentiated by iron addition and greatly reduced by an iron chelator such as deferoxamine (Rottkamp et al. 2001). Metals bound to soluble or aggregated forms of the peptide do seem to be redox active and thus have the capacity to mediate ROS production (Bondy et al. 1998, Huang et al. 1999a,b, Sayre et al. 2000). The A β peptide is considered to be strongly redox active itself and is able to reduce transition metals in the cytoplasm as well as recruiting O $_2$ for the formation of H $_2$ O $_2$ (Huang et al. 1999a). These findings have been replicated in cell culture (Rottkamp et al. 2001) where A β is associated with oxidative damage. However this relationship does not carry over into *in vivo* models where no such relationship exists between rapidly formed oxidative damage and associated pathological markers in AD patients (Nunomura et al. 1999). This difference may be due to the concurrent presence of zinc (Zn $^{2+}$) which is also enriched in these pathological structures and may in fact muse the pro-oxidative action of iron or copper (Cuajungco et al. 2000, Lovell et

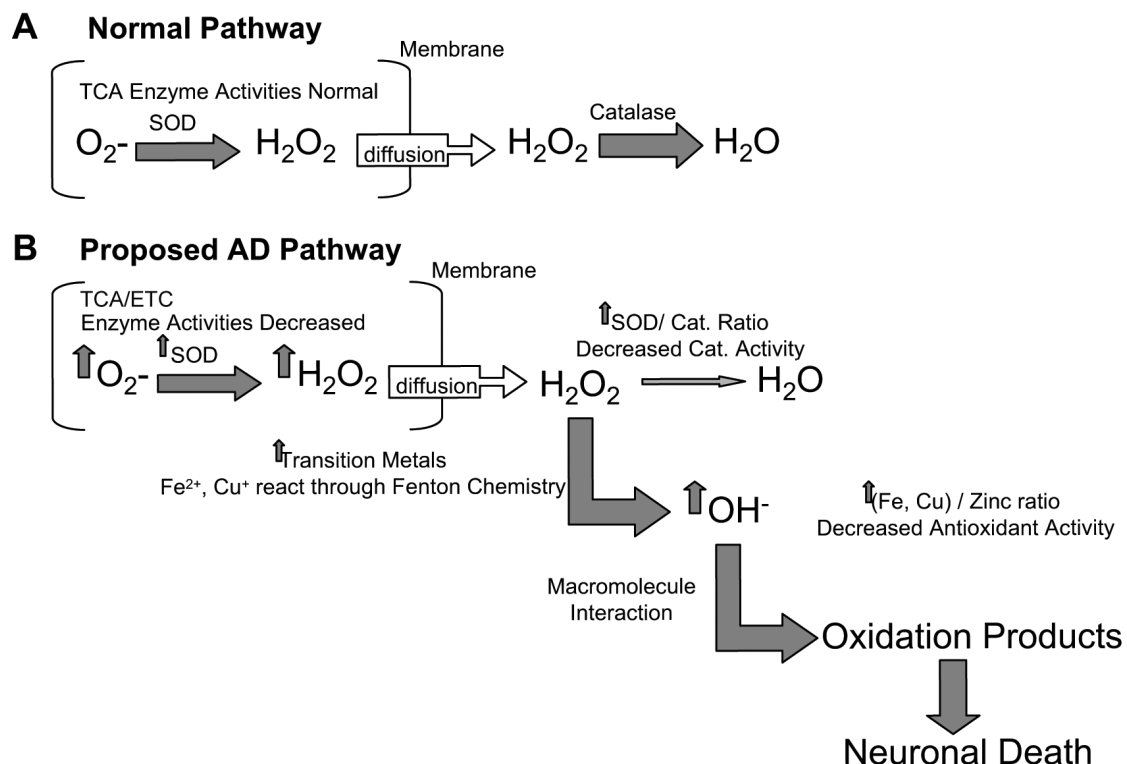


Fig. 1. Schematic illustration of proposed sources and mechanisms involved in cytoplasmic oxidative damage in Alzheimer's disease. (A) Normal pathway. Superoxide is converted to water through action of SOD and catalase. (B) Proposed AD pathway. Deficiency in key mitochondrial enzymes such as COX I and increased SOD activity leads to increased hydrogen peroxide in mitochondria and the surrounding cytoplasm. Decreased catalase/SOD ratio in AD neurons decreases the ability to remove increased hydrogen peroxide, which is then converted to highly reactive hydroxyl radical through Fenton reaction in the presence of enriched iron/copper, thus causing oxidative damage in the cytoplasm.

al. 1998). However, the overall concentration of zinc, an ion with antioxidant activity, is indeed decreased in AD brain (Panayi et al. 2002). Therefore, the enrichment of zinc in pathological structures, although may help to muse the toxic effect of iron/copper in these structures, may further exacerbate the imbalance of pro-oxidative and anti-oxidative factors in the cytoplasm.

CONCLUSIONS

It is evident that oxidative stress plays a significant role in the pathogenesis of AD and multiple evidence showing mitochondrial abnormalities suggest the intimate involvement of mitochondria in the disease. However, the prominent increase in neuronal oxidative damage is restricted to cytoplasm of the susceptible neurons. We suggest that abnormal mitochondria in susceptible neurons act as a source by providing membrane diffusible hydrogen peroxide to the surrounding cytoplasm (Fig. 1). Cytoplasm is more vulnerable to hydrogen peroxide due to the fact that: (i) it is less protected than mitochondria; (ii) catalase/SOD ratio is decreased in AD which decreases the ability to efficiently remove hydrogen peroxide; (iii) enriched pro-oxidative metal ions catalyze the Fenton reaction to produce highly reactive hydroxyl radical (Fig. 1). Therefore, by releasing excess levels of hydrogen peroxide, abnormal mitochondria propagate a series of events involving enriched metal ions and cause damage in cytoplasm.

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