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Compensatory responses induced by oxidative stress in Alzheimer disease

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ABSTRACT

Oxidative stress occurs early in the progression of Alzheimer disease, significantly before the development of the pathologic hallmarks, neurofibrillary tangles and senile plaques. In the first stage of development of the disease, amyloid-β deposition and hyperphosphorylated tau function as compensatory responses and downstream adaptations to ensure that neuronal cells do not succumb to oxidative damage. These findings suggest that Alzheimer disease is associated with a novel balance in oxidant homeostasis.

Key terms: Alzheimer disease, antioxidant therapy, mitochondria, oxidative stress, redox metals

INTRODUCTION

Alzheimer disease (AD) is a common and complex neurodegenerative disorder that affects approximately 15 million people worldwide (Mayeux and Sano, 1999). Aging is the major risk factor for this disease that coexists with other causes of cognitive decline, particularly vascular dementia. The neuropathological hallmarks of AD are synapse abnormalities, intracellular neurofibrillary tangles (NFT) containing mainly polymerized and hyperphosphorylated tau protein, and the accumulation of extracellular amyloid plaques containing mainly amyloid-β (Aβ). AD is devastating, and despite great strides in recent years, its causes and mechanisms are still puzzling. There is accumulating evidence, however, that oxidative stress plays an important role in the disease pathophysiology. While intracellular oxidative balance is tightly regulated, the activation of multiple signaling pathways and the upregulation of compensatory mechanisms therefore would be expected to occur in neurons from AD patients.

Oxidative stress is a prominent event in the development of Alzheimer disease

Historically, efforts to understand oxidative status have focused on the concept of oxidative stress, i.e., the breaching of oxidant defenses with consequent cellular detrimental effects. According to this definition, the detection of damage resulting from reactive oxygen or nitrogen species indicates the existence of oxidative stress. However, this definition fails to consider the ability of living systems to dynamically regulate their defense mechanisms in response to oxidative stress. Therefore, increases in oxidative damage do not indicate that the cell is succumbing to oxidative stress given that the cell may have increased its defenses sufficiently to compensate for the increased flux of reactive species responsible for the damage. This concept is outlined critically by evidence suggesting that cells failing to compensate for oxidative imbalance enter into apoptosis, which in turn leads to death within hours. It thus follows that cells can only experience oxidative stress for short periods of time

before rapidly dying (Perry et al., 1998a; Perry et al., 1998b). In chronic degenerative disorders, such as AD, the cells experiencing increased oxidative damage must develop compensatory responses aimed to avoid or, at least, reduce cellular damage or death.

It has been shown that increased oxidative damage is a prominent and early feature of vulnerable neurons in AD (Smith et al., 1997a; Smith et al., 1997b). Over the past decade, oxidative stress-related modifications of macromolecules have been described in association with the susceptible neurons of AD. Such modifications include advanced glycation end products (Smith et al., 1994), nitration (Smith et al., 1997b), lipid peroxidation adduction products (Montine et al., 1996; Sayre et al., 1997), carbonyl-modified neurofilament protein and free carbonyls, as well as glycation and glycoxidation products (Castellani et al., 2001; Smith et al., 1994; Smith et al., 1995). Levels of these markers are initially elevated following some unknown triggering neuronal event, but these levels soon decrease as the disease progresses to advanced AD (Perry et al., 2002). Together, these findings suggest that increased oxidative damage is not the terminal sequelae of the disease but instead plays an initial role. They also suggest that damage does not mark further destruction by reactive species and is instead marked by a broad array of increased cellular defenses (Perry et al., 2002). It can be argued that in AD these defenses are responsible for the reduction of damage if we view AD in isolation. When seen in the context of other conditions where reactive oxygen and nitrogen species are involved and damage is either limited or absent, the oxidative damage noted in AD may be better thought of as homeostatic, i.e., oxidative damage could initiate signal transduction pathways that manipulate cellular responses to stress, which are characterized by increased levels of reactive oxygen and nitrogen species (Perry et al., 2002).

Mitochondria is one major source of oxidative stress

Extensive evidence indicates that cerebral metabolism is reduced in AD (Kalaria et al., 1988), placing mitochondria as a key player in this scenario (Hirai et al., 2001). Mitochondria are essential organelles for neuronal function because the limited glycolytic capacity of these cells makes them highly dependent on aerobic oxidative phosphorylation for their energetic needs. However, oxidative phosphorylation is a major source of endogenous, toxic-free radicals, including hydrogen peroxide (H_2O_2), hydroxyl ($\bullet OH$) and superoxide ($O_2^-\bullet$) that are products of normal cellular respiration (Wallace, 1999). Reactive species generated by mitochondria have several cellular targets including mitochondrial components themselves (lipids, proteins, and DNA). The lack of histones in mitochondrial DNA (mtDNA) and diminished capacity for DNA repair render mitochondria an easy target for oxidative stress events. By releasing high levels of H_2O_2, dysfunctional mitochondria promote the interaction between redox metals and oxidative response elements (Zhu et al., 2004). The role of mitochondria in AD were studied in our laboratory using *in situ* hybridization to mitochondrial DNA, immunocytochemistry of cytochrome oxidase, and morphometry of electron micrographs of biopsy specimens to characterize the mitochondrial abnormalities in AD (Hirai et al., 2001; Castellani et al., 2002). We found that the neurons showing increased oxidative damage in AD also possess a striking and significant increase in mtDNA and cytochrome oxidase. Surprisingly, much of the mtDNA and cytochrome oxidase is found in the neuronal cytoplasm, and in the case of mtDNA, the vacuoles associated with lipofuscin, whereas morphometric analysis showed that mitochondria are significantly reduced in AD. We also observed an overall reduction in microtubules in AD compared to controls (Cash et al., 2003). Altogether, these data indicate that the abnormal mitochondrial turnover, as indicated by increased perikaryal mtDNA and mitochondrial protein accumulation in the face of reduced numbers of mitochondria, could be due to a defective microtubule metabolism resulting in deficient mitochondrial transport in the axon. This may, in turn, set up a pathological cascade of events in the perikaryal mitochondria.

Accumulating evidence indicate that heme oxygenase-1 (HO-1) is induced in AD brains (Premkumar et al., 1995; Takeda et al., 2004). HO-1 catalyzes the conversion of heme to biliverdin and iron. Biliverdin, in turn, is reduced to bilirubin, an antioxidant. Since HO-1 is induced in proportion to the level of heme (Keyse and Tyrrell, 1989), the induction of HO-1 suggests that there may be abnormal turnover of heme in AD. This idea is consistent with the mitochondrial abnormalities associated with AD since it is well known that many heme-containing enzymes are found in mitochondria. As previously discussed, our ultrastructural studies centered on mitochondria suggest a high rate of mitochondrial turnover and redox activity in the residual body of lipofuscin (Hirai et al., 2001), with lysosomes the probable sources of heme. In turn, the increase in heme induces synthesis of more HO-1, suggesting that mitochondrial turnover promotes oxidative stress via increases of redox-active iron (Honda et al., 2004).

Furthermore, we observed that oxidized nucleic acids are commonly observed in the cytoplasm of the neurons that are particularly vulnerable to degeneration in sporadic AD (Nunomura et al., 1999b; Nunomura et al., 2001) and familial AD (Nunomura et al., 2004), which suggest a link between oxidative stress and not only age-associated degenerative diseases but also neurodegeneration linked to genetic factors. 8OHG, a marker of nucleic acids oxidation, is likely to form at the site of •OH production, a process dependent on redox-active, metal-catalyzed reduction of H_2O_2, together with cellular reductants such as ascorbate or O_2^-. Interestingly, the levels of 8OHG are inversely related to the extent of Aβ deposits (Nunomura et al., 1999b), suggesting a complex interplay between Aβ and redox metal activity that may be critical to metal dynamics within the neuronal cytoplasm. A possible key element linking these dynamics is mitochondria within neuronal cells.

Oxidative Stress Induces Cellular Compensatory Responses

Oxidative modification and response are controlled events that play critical homeostatic roles as a reaction to oxidative stress or even more fundamental abnormalities. This view is supported by data comparing AD with normal aging, where we documented the same profile of damage, albeit with lower levels (Nunomura et al., 1999b). AD differs from other neurodegenerative diseases in that it shows exponential prevalence with aging. This makes AD a part of the normal aging process, which encompasses additional specific pathological mechanisms and suggests that many of the oxidative modifications resulting from aging also play a role in balancing systems underlying the metabolic abnormalities present in AD. Support for this idea comes from the analysis of lipid peroxidative modification of tau and neurofilament heavy subunit (NFH), two of the components of neurofibrillary tangles (Perry et al., 1985). Additionally, it was suggested that NFT are antioxidants since both tau and neurofilament protein appear uniquely adapted to oxidative attack, due to their high content of lysine-serine-proline domains (Wataya et al., 2002). Exposure of these domains on the surface is affected by extensive phosphorylation of serine residues (Wataya et al., 2002; Liu et al., 2005). In AD, both tau and NFH are found as adducts of HNE, and the modifications are controlled by phosphorylation (Takeda et al., 2000b; Wataya et al., 2002). While NFH is modified by HNE at similar levels in AD and controls (Perry and Smith, unpublished data), normal brains present only small tau modifications (Takeda et al., 2000b). Tau modification by HNE greatly increases its ability to form filaments –*in vitro* (Pérez et al., 2000) and in cells (Pérez et al., 2002)– similar to those found in NFT. The link to phosphorylation and the known similarity of oxidative imbalance to induction of the MAP kinase pathway (Perry et al., 1999; Zhu et al., 2001b; Zhu et al., 2001a; Zhu et al., 2004) suggest that lipid peroxidation modification of tau is regulated by signal transduction pathways. Studies performed with rodents show that NFH-HNE levels do not increase with aging or movement of NFH to terminals, suggesting that levels of adduction are further regulated by removal

or reversal of NFH-HNE adducts (Wataya et al., 2002). These data indicate this process may play a physiological role in axons, possibly protecting axonal components by scavenging toxic aldehydes formed during normal metabolism. This would be essential for axons, which cannot turn over and replace proteins as rapidly as cell bodies.

Transfection studies of neuroblastoma cells show that HO-1 expression is coordinated with tau phosphorylation, suggesting a physiological interaction between cytoskeleton reorganization and oxidation (Takeda et al., 2000a). In consideration of the enzymatic activity of HO-1 to produce iron and tau to bind iron (Sayre et al., 2000), the coordination may relate to iron homeostasis. These results clearly implicate a regulated process for modifications and responses. Changes such as HO-1 and MAP kinase pathway induction may be only a few of the many responses that interrelate to lipid peroxidative modifications. In this way, oxidative damage is no longer an end stage but rather a signal of underlying changes of state.

Furthermore, an antioxidant role for Aβ *in vivo* is in agreement with recent data on the distribution of oxidative damage to AD neurons. 8-Hydroxyguanosine (8OHG), a major product of nucleic acid oxidation, markedly accumulates in the cytoplasm of cerebral neurons in AD. Unexpectedly, an increase in Aβ deposition in AD cortex is associated with a decrease in neuronal level of 8OHG, i.e., with decreased oxidative damage (Nunomura et al., 1999a; Nunomura et al., 1999b). Similar negative correlation between Aβ deposition and oxidative damage is found in patients with Down syndrome (Nunomura et al., 2000). Diffuse amyloid plaques may be considered as a compensatory response that reduces oxidative stress (Smith et al., 2000). The protective properties of Aβ could be related to its capacity to scavenge free radicals and the chelation of transition metals (Lovell et al., 1998; Unnikrishnan and Rao, 1990).

Altogether these findings suggest that both Aβ and NFT, in the initial stages of AD development, may be compensations to combat increased oxidative stress.

Oxidative stress should be the target of future therapeutic strategies

Given the proximal role and devastating effect that oxidative stress plays in AD pathogenesis, a therapeutic strategy based on fighting oxidative stress appears reasonable. Certainly, such an assumption should be considered more reasonable than strategies based either on removal of Aβ or on preventing the phosphorylation of tau, since both represent downstream consequences of oxidative stress and/or a concerted cellular effort to maintain homeostatic balance. In addition, data from the literature show that strategies envisaging the removal of Aβ deposits (immunotherapy) have severe side effects, such as the activation of central nervous system inflammatory responses (Monsonego et al., 2003; Nicoll et al., 2003).

There is overwhelming clinical evidence that antioxidants, free radical fighters, slow the symptoms of both dementia and AD. Although modest, several epidemiological studies have shown lower risks of dementia and AD progression in people taking dietary antioxidants (Sano, 2003). However, the broad occurrence of AD (almost 50% by the age of 85), the non-regenerative nature of the CNS, and the fact that diagnosis often does not occur until late in disease progression, suggest that the ideal antioxidant should be used as prophylactic treatment in the aged population. Due to their low toxicity, low cost and their ability to target the earliest sources of oxidative stress in AD, antioxidant therapies are particularly attractive.

CONCLUSIONS

Instead of viewing oxidative stress as the breach of antioxidant defenses, we argue that this seldom happens in chronic conditions, pathological and physiological, and that a better understanding occurs by viewing each circumstance as a different, homeostatic balance in which reactive oxygen and nitrogen species play a key regulatory role. In the initial stages of AD development, neuronal cells, despite showing increased oxidative damage, may actually be in homeostatic balance. If cells

survive and function in the presence of high levels of oxidative stress, it is because critical systems of cells are not damaged. In this way, detection of increased oxidative damage in cells that survive must be associated with a commensurate increase in compensatory mechanisms such as Aβ deposition and hyperphosphorylated tau. However, with the progression of AD and the consequent increase of reactive species levels, the efficient removal of Aβ-metal complexes and, probably, hyperphosphorylated tau would be overtaken by their disproportionably high generation, resulting in an uncontrollable growth of plaques and NFT and, consequently, an increase in reactive species generation. This would result in a feedback mechanism that could exacerbate plaque and NFT growth and reactive species generation, leading to a functional demise of neurons (Fig. 1).

Oxidative stress

Fe
Cu

Compensatory responses

Oxidative stress overwhelms the compensatory responses
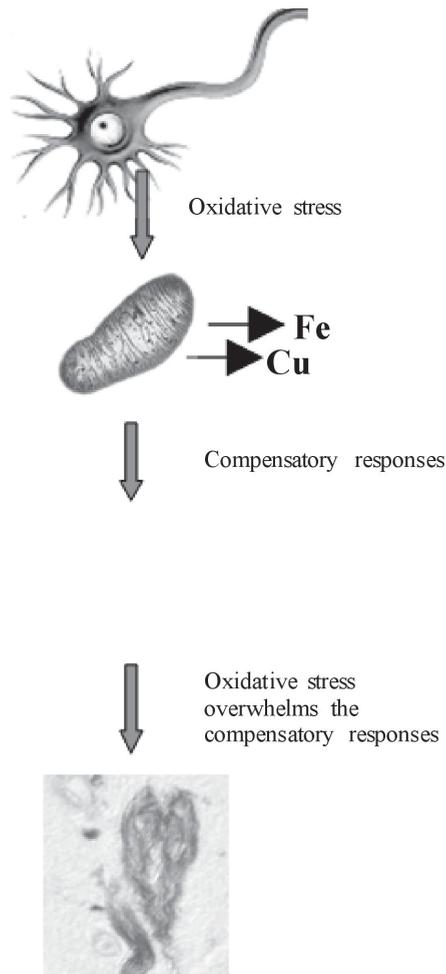
Neuronal damage and death

Figure 1. Possible sequence of neuronal events that occurs in AD. In the initial stages of AD, the increase in oxidative stress levels, mainly promoted by the interplay between the reactive species formed by mitochondria and the transition metals, induce neuronal compensatory responses and downstream adaptations, such as MAP kinase activation, Aβ deposition and tau hyperphosphorylation, aimed to avoid neuronal damage and/or death. However, with the progression of AD and the consequent increase of reactive species levels, the efficient removal of Aβ-metal complexes and, probably, hyperphosphorylated tau would be overtaken by their disproportionably high generation, resulting in uncontrollable growth of plaques and NFT and consequently, an increase in reactive species generation. The increase in oxidative agents results, inevitably, in neuronal damage and death.

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