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Review

Comparative biology and pathology of oxidative stress in Alzheimer and other neurodegenerative diseases: beyond damage and response☆

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Abstract

In this review, we consider comparative aspects of the biology and pathology of oxygen radicals in neurodegenerative disease and how these findings have influenced our concept of oxidative stress. The common definition of oxidative stress is a breach of antioxidant defenses by oxygen radicals leading to damage to critical molecules and disrupted physiology. Inherent in this definition is that oxidative stress is an unstable situation, for if there is net damage, viability of the system decreases with time, leading to disequilibria and death. While this circumstance defines acute conditions, such as stroke and head trauma which result in dysfunction and death, it does not fit physiological situations or chronic diseases closely aligned to normal physiology. Therefore, we propose that oxidative modifications in Alzheimer disease may actually serve as a homeostatic response to stress resulting in a shift of neuronal priority from normal function to basic survival. This phenomenon is comparable to normal physiological conditions of metabolic decrease, such as those seen in hibernation and estivation. Thus, Alzheimer disease could be seen as part of normal aging that includes additional pathology due to inadequate homeostatic response.

Keywords: Aging; Alzheimer disease; Hibernation; Homeostasis; Iron; Mitochondria; Neurodegenerative disease; Oxidative damage; Reactive oxygen species

In Alzheimer disease (AD), one sees damage to every category of macromolecule, specifically in the cytoplasm of vulnerable neurons (Table 1). This damage is an early event, occurring prior to the cytopathology of AD and in fact lessening with its progression (Nunomura et al., 2001). Fig. 1 illustrates this phenomenon using nitrotyrosine, lipid peroxidation, and 8-hydroxyguanosine, three distinct and measurable markers of oxidative damage in AD. Levels of these markers are initially elevated following some unknown triggering neuronal event, but these levels soon decrease as the

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disease progresses to advanced AD. These findings suggest that increased oxidative damage is not the terminal sequelae of disease but instead plays an initial role. They also suggest that damage does not mark further destruction by reactive oxygen species (ROS) and is instead marked by a broad array of increased cellular defenses (Table 2). It can be argued that in AD these defenses are responsible for the reduction of damage if we view AD in isolation. When instead seen in the context of other conditions where ROS are involved and damage is either limited or absent, such as Parkinson disease (Zhang et al., 1999), this result leads us to consider whether oxidative damage noted in AD may be better thought of as homeostatic, i.e., that oxidative damage could initiate signal transduction pathways to manipulate cellular responses to stress, which is characterized by increased levels of ROS.

Production of ROS throughout cells and tissues normally occurs as a byproduct of oxidative phosphorylation and oxidases which support aerobic metabolism. In AD, there are a number of additional ROS sources which can play a role in the disease process: (1) redox-active iron, at increased levels in tangles and plaques, can catalyze the formation of $^{\bullet}OH$ from H_2O_2 as well as the formation of advanced glycation endproducts (AGE); (2) activated microglia, such as those surrounding senile plaques, can produce nitric oxide and O_2^-, which can subsequently react to form peroxynitrite, with nitrotyrosine as the identifiable marker; (3) amyloid-β has been directly implicated in ROS formation by means of peptidyl radicals; (4) AGE, in the presence of redox-active metals such as iron, can undergo redox cycling resulting in ROS production. AGE can further increase ROS formation by activation of specific receptors, such as the receptor for AGE (RAGE) and the class A scavenger-receptor. Amyloid-β is also capable of this receptor activation; and (5) mitochondrial metabolic abnormalities, such as changes in the mitochondrial genome or deficiencies in key metabolic enzymes, may be a major initiating source of ROS in AD (Perry et al., 1998c).

Examination of a number of neurodegenerative diseases where oxidative stress has been implicated show quite distinct patterns of damage ranging from AD, where every category of macromolecule shows modification, to ALS, where damage is not significantly increased above the high levels seen in normal motor neurons (Perry and Smith, unpublished observations), to progressive supranuclear palsy (PSP), where damage is restricted to lipid prooxidation (Odetti et al., 2000). These distinctions suggest that the responses to different disease processes yield quite distinct profiles of damage rather than stereotypical global changes. Why the profiles are so different among diseases has concerned us for years, leading us initially to think that oxidative stress may not be involved in all of these diseases or that damage shows restrictive compartmentation among diseases.

Table 1
Targets of oxidative damage in Alzheimer disease

Macromolecule	Type of damage
Sugars	Glycation
Protein	Carbonyls, nitration
Lipids	Lipid peroxidation
Nucleic acid	8-hydroxyguanosine

More recent findings suggest, what we think is a more exciting possibility, that 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 reduced levels (Nunomura et al., 1999). AD differs from all the other diseases in that it shows exponential increase with aging, with approximately half of all individuals afflicted by the age of 95 (US General Accounting Office, GAO/HEHS-98-16). This makes AD a part of the normal aging process which encompasses additional specific pathological mechanisms and suggests that many of the oxidative modifications may not only result from aging but also play a role in balancing systems underlying the metabolic abnormalities present in AD.

Support for this idea comes from analysis of lipid peroxidative modification of tau and neurofilament heavy subunit (NFH), two of the components of neurofibrillary tangles (Perry et al., 1985). In AD, both proteins are found as adducts of hydroxynonenal (HNE), a highly reactive aldehyde resulting from lipid peroxidation. The modifications are lysine specific and, for both proteins, controlled by phosphorylation (Takeda et al., 2000a; Wataya et al., 2002) leading to different patterns vis-à-vis AD/controls. While NFH is

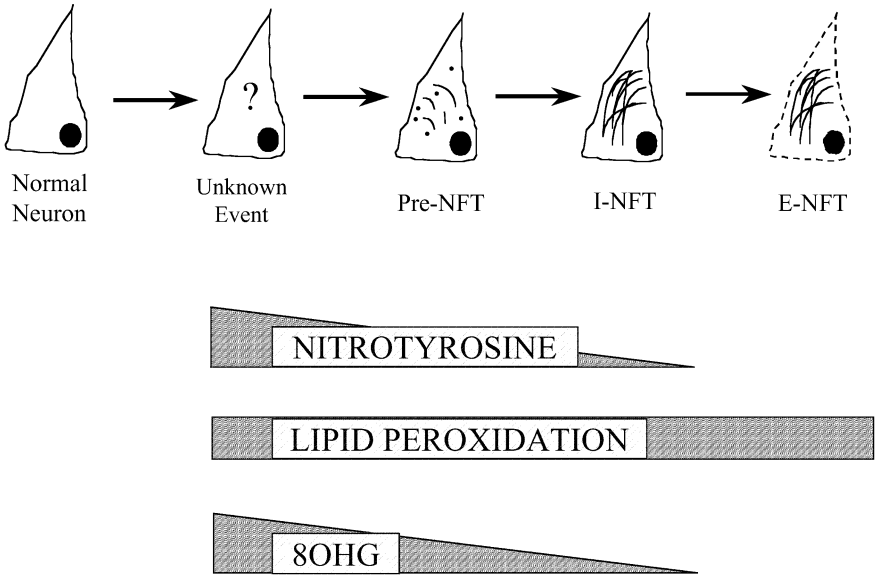

Fig. 1. Time course of neuronal degenerative changes resulting in oxidative damage in AD. Oxidative modifications are found in all vulnerable neurons even before neurofibrillary tangles are formed.

normally phosphorylated as it enters the axon, in AD phosphorylation occurs in the cell body (Sternberger et al., 1985). In contrast, tau is more extensively phosphorylated in AD (Grundke-Iqbal et al., 1986). Thus, while NFH is modified by HNE at similar levels in AD and controls (Perry and Smith, unpublished data), for tau little is modified in normal brain (Takeda et al., 2000a). Tau modification by HNE greatly increases its ability to form filaments, in vitro (Perez et al., 2000) and in cells (Pérez et al., 2002), similar to those found in neurofibrillary tangles (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., 2000, 2001a,b,c) suggest that lipid peroxidative modification of tau is regulated by signal transduction pathways. In the case of NFH, its normal traversal from the cell body to nerve terminals is associated with phosphorylation in the proximal axon. The significance of NFH modification by HNE with phosphorylation requires further study, but observation in rodents shows that NFH–HNE levels do not increase with aging or

Table 2
Cellular responses to oxygen radicals in Alzheimer disease

System	Effect
Heme oxygenase increase	Conversion of prooxidant heme to antioxidant bilirubin and iron
Glucose 6 phosphate dehydrogenase increase	Rate limiting step of pentose phosphatase pathway, primary pathway of reducing equivalent
Reduced sulfhydryls	Increased level of −SH groups such as glutathione play critical roles in removing toxic products
MAPkinase activation	Signal transduction pathways are linked to oxidative stress by phosphorylating critical residues
Neurofilaments	Major target of lipid adduction in the neuron. Adduction controlled by phosphorylation
Tau	Modified by hydroxynonenal in AD, modification controlled by phosphorylation
Amyloid β	While capable of causing metal catalyzed reactive oxygen formation in vitro, is associated with decreased oxidative damage in vivo

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). That NFH is normally modified, even regulated to do so, suggests that this process may play a physiological role in axons, possibly in protecting axonal components by scavenging toxic aldehydes formed during normal metabolism. This would be essential for axons, which cannot turn over proteins as rapidly as cell bodies.

Phosphorylation and lipid peroxidation of tau are marked by changes leading to fibril formation as well as by epitopic changes in tau that define AD. In the case of the epitope recognized by the antibody Alz50, formation is controlled by a reaction with HNE to phosphorylated tau (Takeda et al., 2000a). Coincident and topographically identical are the Alz50 epitope and heme oxygenase 1 (HO-1), a known antioxidant enzyme. In transfection studies of neuroblastoma cells, HO-1 expression was coordinated with tau phosphorylation, suggesting again a physiological interaction (Takeda et al., 2000b). In consideration of the enzymatic activity of HO-1 to produce iron and of tau to bind iron (Perez et al., 1998; Sayre et al., 2000), the coordination may relate to iron homeostasis. While these observations do not establish the physiological significance of lipid peroxidative modification, they clearly implicate a regulated process for modifications and response. Changes such as HO-1, MAP kinase, and pentose phosphate pathway induction, as well as antioxidant responses, may be only a few of many responses that interrelate to lipid peroxidative modification. Seen as such, oxidative damage is no longer an end stage event but rather a signal of an underlying change of state.

Many lines of evidence point to a fundamental diminution of metabolism as the initiation of AD. Imaging studies of patients genetically at risk of AD show diminished metabolism 20 years prior to dementia (Small et al., 1996). Diminished glucose transporter (GLUT-1) levels in cerebral vessels also mark AD (Kalaria et al., 1988). While neuronal oxidative damage occurs early in AD, oxidative damage to endothelial cells is seen only in cases of long duration, suggesting that vascular changes are not early events but rather atrophy reflecting lowered demand (Aliev et al., 2002; Nunomura et al., unpublished data). When we examined specimens removed at biopsy from patients with AD and controls, we found a slight decrease in mitochondrial density (Hirai et al., 2001). What was most significant was the accumulation of mtDNA in autophagocytes, as well as mitochondrial proteins and iron in the cytoplasm. Analysis of other organelles showed significant changes only in lipofuscin/autophagy, an increase likely related in part to the mitochondrial abnormalities, and a reduction by half of microtubules, microtubules being the track on which mitochondria traverse the axon (Cash and Perry, unpublished observations). Coordinate reduction in microtubules, increased autophagy of mitochondria, and disruption of the Golgi (Mourelatos et al., 1996; Stieber et al., 1996) indicate a likely re routing of cellular metabolism away from supporting axons (Fig. 2), resulting in reduced synaptic vesicle transport and leading to the dementia defining AD (Terry, 1996).

Preliminary analysis of aged subjects shows that diminished microtubule density correlates with decreased Na/K ATPase (Hattori et al., 1998) and induction of the pentose phosphate pathway (Russell et al., 1999). These findings point to a fundamental lowering of energy production and a shift in its utilization away from normal function in transport and ion balance to increased defenses. Without these changes, we argue that neurons would succumb to apoptosis, as release of mitochondrial components is typically followed by death in control neurons. In contrast, neuronal death characterizing AD is slow and inconsistent (Perry et al., 1998a,b, 2001; Marx, 2001).

We hypothesize that the changes of AD may mark a major shift in neuronal priority from function to survival by normal physiological terms. Human brain metabolism is dictated by high neuronal function, as indicated by the utilization of 20–25% of basal metabolism by the brain. This is particularly striking because the brain constitutes approximately 2% of the body and neurons have a metabolism 5 times that of other brain cells. The reduction in brain metabolism with AD therefore necessitates new neuronal energetic priorities.

We further suggest that these changes in priorities may be analogous to those of hibernation, where brain energy demands are strikingly reduced as compensation for decreased energy supply. During this period, neurons are preserved under conditions of low energy expenditures, which can cause massive neuronal death if acutely encountered as in stroke. Instead, in hibernation neurons are resistant to damage from this type of trauma

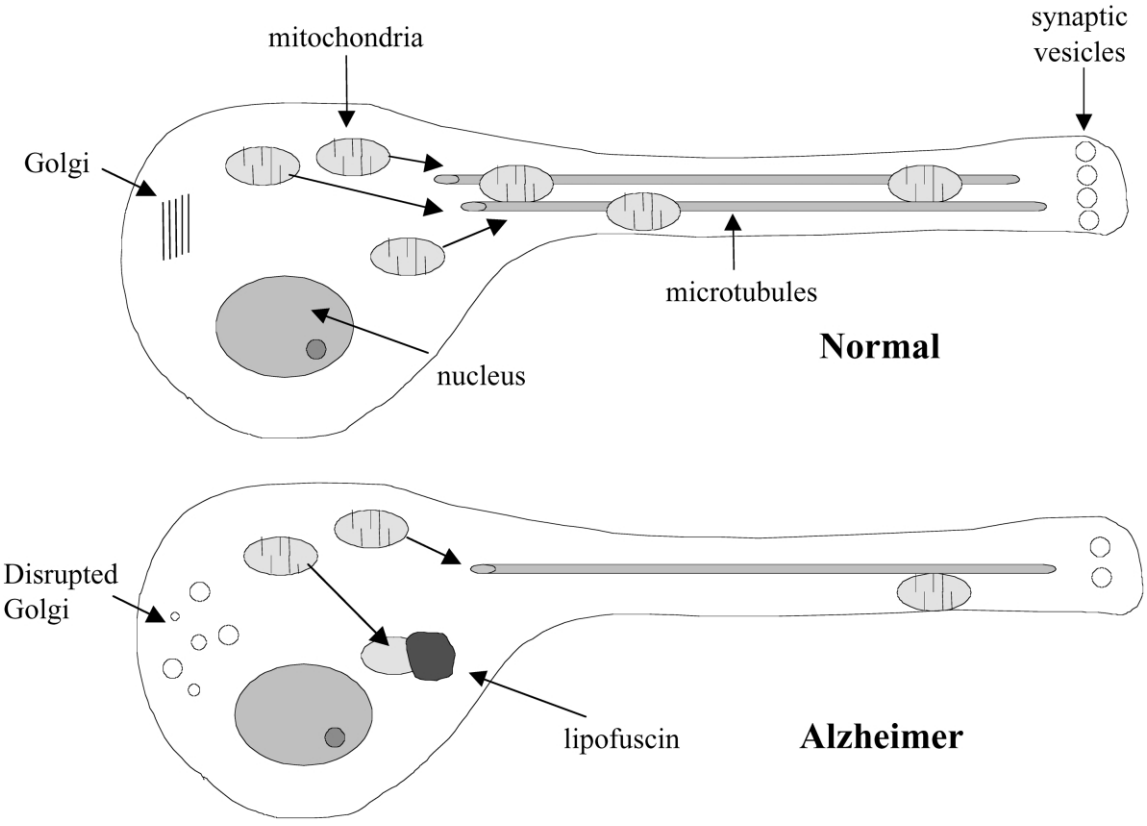

Fig. 2. We hypothesize that diminished in vesicular transport in AD due to reduced microtubules leads to a decrease in axonal mitochondria, with increased turnover in the cell body. Also associated is disruption of the Golgi and reduction of synaptic vesicles.

(Zhou et al., 2001). Could AD be the human brain's attempt to compensate for decreased energy supply; an attempt to decrease energy demands while waiting for a Spring that will never come? In order for human brains to down regulate metabolic demand, a novel homeostatic balance must be established.

It has been demonstrated that soluble amyloid-β is capable of mediating a metabolic switch to inhibit glucose usage. These processes are initiated in normal aging and include inhibition of a number of hormones and neurotransmitters, which are key regulators of glucose metabolism, such as proglycolytic neurohormones and pyruvate dehydrogenase. In turn, levels of hormones and effector systems, such as nitric oxide and calcium ions, are increased. The resulting energetic insufficiency results in a shift of neuronal activity away from axons toward cell bodies, which eventually leads to degeneration of vulnerable neurons (Heininger, 2000). These findings encourage a compensatory homeostatic role for amyloid-β, whose role in AD is presently unclear. Similarly, oxidative damage instead of being the culprit in AD may, in reality, play an important regulatory role in this process.

Oxidative changes seen in normal aging are similar to oxidative changes seen in AD (Perry and Smith, unpublished observation). The time and extent of oxidative changes overlap in both aging and AD, arguing that the regulatory changes can occur without a loss of function, that they can be successful (normal aging) as well as unsuccessful (AD) in maintaining both function and normal viability (Guarente and Kenyon, 2000; Gems and Partridge, 2001; Kenyon, 2001). One possible method of energy regulation is oxidation-induced inactivation of key respiratory chain complexes. Inactivation of these enzymes could result in a decrease in energy production and might be responsible for ATP depletion. Our hypothesis suggests that restoring and maintaining high metabolism with aging would be associated with reduction in AD, but it also suggests that therapeutics directed towards maintaining energy balance

will have great efficacy in AD if applied before compensatory, homeostatic mechanisms down regulate metabolic demand to the point of no return.

These findings bear not only on AD but also on other normal physiological conditions where metabolism is intermittently decreased, such as in hibernation and estivation. The expectation that these conditions would be met with oxidative modification and response has not been substantiated. Instead these uniquely adapted animals maintain homeostasis without the pleiotrophic changes of AD. We hypothesize that the absence of demonstrated oxidative damage in many of these animals does not mean that there is more or less ROS flux present than in AD, only that the outcome of that flux is not associated with induction of the massive changes noted in AD. Instead of viewing oxidative stress as the breach of defenses, we argue this seldom happens in chronic conditions, pathological and physiological, and that a better understanding is made by viewing each circumstance as a different homeostatic balance in which ROS plays a key regulatory role. This model requires greater understanding than simply knowing what causes ROS and which molecules are damaged, but further exploration of this model is likely to yield the greatest comprehension of the physiology and pathology of oxidative phenomena.

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