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Biochimica et Biophysica Acta 1502 (2000) 139–144

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Review

Oxidative stress in Alzheimer's disease

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Received 15 November 1999; received in revised form 7 March 2000; accepted 7 March 2000

Abstract

Oxidative balance is emerging as an important issue in understanding the pathogenesis of Alzheimer's disease. Examination of Alzheimer's disease brain has demonstrated a great deal of oxidative damage, associated with both hallmark pathologies (senile plaques and neurofibrillary tangles) as well as in normal appearing pyramidal neurons. While this suggests that oxidative stress is a proximal event in Alzheimer's disease pathogenesis, the mechanisms by which redox balance is altered in the disease remains elusive. Determining which of the proposed sources of free radicals, which include mitochondrial dysfunction, amyloid-β-mediated processes, transition metal accumulation and genetic factors like apolipoprotein E and presenilins, is responsible for redox imbalance will lead to a better understanding of Alzheimer's disease pathogenesis and novel therapeutic approaches.

Keywords: Antioxidant; Amyloid-β; Free radical; Mitochondria; Redox imbalance; Transition metal

1. Introduction

For Alzheimer's disease (AD), the majority of research resources have been dedicated to studies on the pathogenesis of the intraneuronal filamentous inclusions, known as neurofibrillary tangles (NFT), and the extracellular senile plaques. This focus has often been detrimental to the advancement of other theories. Consequently, there is a large void in our understanding of the pathogenesis of AD, namely the underlying mechanism of the disease. Nonetheless, in recent years, research has clearly pointed to the importance of oxidative imbalance in AD. Here, we review some of these fundamental insights that have indicated the importance of oxidative stress and free radical damage to the pathogenesis of AD.

2. Oxidative damage

Under normal conditions, damage by oxygen radicals is kept in check by an efficient array of antioxidant systems that display extensive redundancy (e.g. the simultaneous metabolism of H_2O_2 by catalase and glutathione peroxidase). However, during pathological conditions, the oxidant versus antioxidant balance is necessarily altered, either primarily or secondarily. Oxidative damage occurs when the oxidative balance is disturbed such that reactive oxygen production exceeds cellular anti-oxidant defenses. The oxidative damage found in AD includes advanced glycation end products [1–4], nitration [5,6],

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0925-4439/00/$ – see front matter
PII: S0925-4439(00)00040-5

lipid peroxidation adduction products [7,8], carbonyl-modified neurofilament protein and free carbonyls [9–11] (Fig. 1). While not necessarily pathogenic, as discussed below, it is important to realize that this damage selectively involves all neurons in populations vulnerable to death in AD.

2.1. Oxidative damage, neurofibrillary tangles and senile plaques

Examination of the spatio-temporal relationship between the presence of oxidative modification and the hallmark lesions of AD reveal a paradox. We found that stable glycation products are predominantly associated with NFT and Aβ deposits [1], whereas reversible or rapidly degraded adduction products are predominately in the cytoplasm of vulnerable neurons. In fact, damage to short-lived molecules appears to be restricted to cytosolic compartments.

In order to address where reactive oxygen is produced, we focused our efforts on finding a marker that results from primary attack and which involves damage to a cell constituent with a short half-life. This allows for the examination of events that proceed those detected by more complex secondary reactions. Proteins fail in the latter aspect of the two criteria mentioned because modifications associated with crosslinking slow their turnover. Therefore, crosslink modifications of proteins, while useful to assess history, may reveal less of the current state. Detection of 8-hydroxyguanosine (8OHG), a nucleic acid modification predominantly derived from hydroxide (•OH) attack of guanidine, allows for assessment of more immediate oxidative damage. 8OHG has been found to be greatly increased in the cytoplasmic RNA of vulnerable neuronal populations [12]. Likewise, neurons containing NFT have extremely low levels of 8OHG (i.e. current oxidative stress status) despite an obvious history of oxidative damage (i.e. advanced glycation endproducts or lipid peroxidation). Notably, however, cases of AD with the most extensive amyloid-β (Aβ) deposits show the lowest levels of 8OHG. These findings seem markedly contradictory considering reports that Aβ is a major source of oxidative free radicals and/or a toxic agent in AD [13] and instead suggests that both Aβ and NFT may be cellular compensations for increased oxidative stress and serve antioxidant functions.

The hypothesis that Aβ plays an antioxidant function is supported by a study of Down's syndrome patients, a disease where Aβ deposits begin in the late teens and in which oxidative stress has been implicated [14]. As with AD, in Down's cases, Aβ deposition follows, rather than precedes, increased 8OHG and 8OHG levels decline to control levels ($r = 0.98$) after Aβ plaques form. Therefore, given

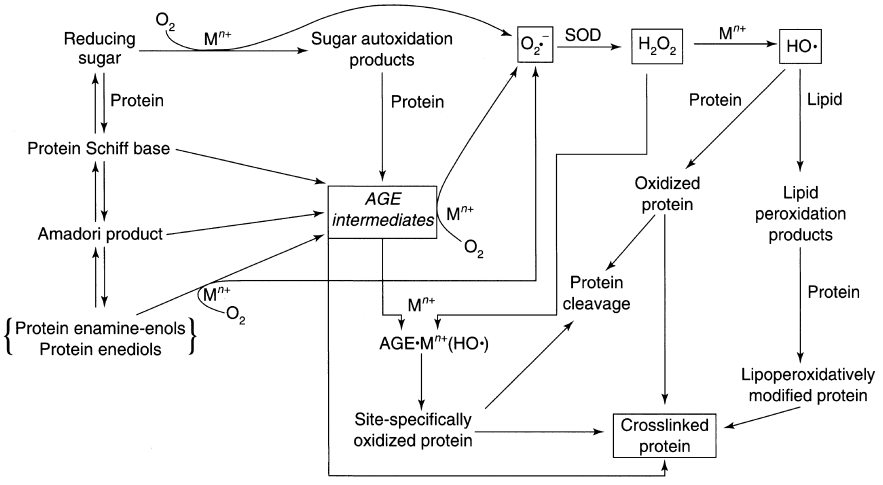

Fig. 1. Schematic showing oxidative-mediated protein modifications are generated via free radicals and oxidative intermediates. Proteins can be modified and crosslinked by glycation, autoxidation and reactive aldehydes. These processes are largely synergistic such that the protein modifications involve production of redox-active species that potentiate free radical autoxidation. (Reproduced with permission from Smith et al., Trends Neuroscience 18(4) (1995) 172–176.)

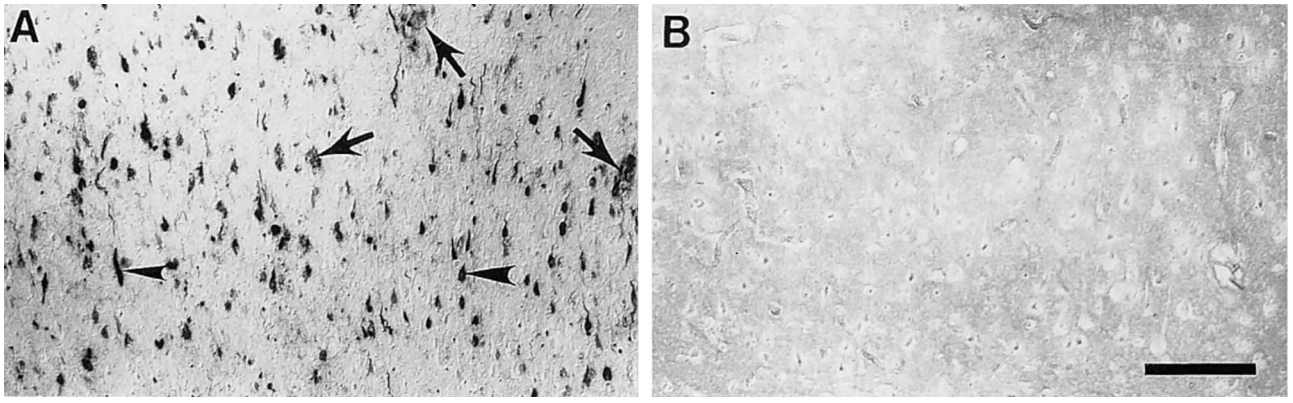

Fig. 2. Redox-active iron in AD brain (A) is strongly associated with the hallmark pathologies, namely neurofibrillary tangles (arrowheads) and senile plaques (arrows). By marked contrast, there is little iron deposition in age-matched control brain (B). Scale bar: 200 μm. (Reproduced with permission from Smith et al. [16].)

that oxidative damage occurs prior to the appearance of other abnormalities, it is unlikely that Aβ, advanced glycation endproducts or infiltration of microglia are primary contributors. However, redox-active iron, especially in conjunction with mitochondrial abnormalities, represent an early and, equally importantly, cytoplasmic base for the generation of oxidizing species.

3. Sources of oxidative stress in Alzheimer's disease

In AD, there are a number of contributory sources that are thought to play an important role in free radical production. (1) Iron, in a redox-active state, is increased in NFT as well as in Aβ deposits [15,16] (Fig. 2). Iron catalyzes the formation of $^{\bullet}OH$ from H_2O_2 as well as the formation of advanced glycation end products. Furthermore, aluminum, which also accumulates in NFT-containing neurons [15], stimulates iron-induced lipid peroxidation [17]. (2) Activated microglia, such as those that surround most senile plaques [18], are a source of NO and $O_2^{-\bullet}$ [19] that can react to form peroxynitrite, leaving nitrotyrosine as an identifiable marker [5,6]. (3) Aβ, itself, has been directly implicated in reactive oxygen formation through peptidyl radicals [13,20,21]. (4) Advanced glycation end products in the presence of transition metals can undergo redox cycling with consequent production of reactive oxygen [4,22,23]. Additionally, advanced glycation end products, as well as Aβ, activate specific receptors, such as the receptor for advanced glycation end products (RAGE) and the class A scavenger-receptor, to increase reactive oxygen production [24,25]. (5) Abnormalities in the mitochondrial genome [26,27] or deficiencies in key metabolic enzymes [28–32] suggest that metabolic abnormalities affecting mitochondria may be the major, and possibly initiating, source of reactive oxygen in AD.

Quantitative analysis of the co-localization of mtDNA deletions and 8OHG in AD cases demonstrates a strong positive correlation ($r = 0.934$). However, mitochondrial DNA, even that which contains the 5 kb deletion, is relatively spared from oxidative damage (i.e. the formation of 8OHG) in comparison to cytoplasmic nucleic acid. We therefore suspect that mitochondrial abnormalities correlate with, but do not directly cause, reactive oxygen. This may be due to the fact that hydroxide radicals, which are responsible for the formation of 8OHG, tend to be fairly short-lived in solution and have a sphere of diffusion of only 2 nm. Therefore, since damage is topographically distinct, it is likely that $^{\bullet}OH$ radical formation occurs in the cytoplasm rather than the mitochondria and that mtDNA is relatively spared due to their inability to diffuse through the mitochondrial membrane. However, abnormal mitochondria may produce excess H_2O_2 through conversion of O_2^- by mitochondrial superoxide dismutase. Such H_2O_2 is readily diffusible and relatively stable, that is, until confronting redox-active transition metals where Fenton chemistry drives the production of $^{\bullet}OH$.

Recent histochemical studies have demonstrated that the direct detection of redox activity in lesions of AD is inhibited by prior exposure of tissue sections to copper and iron selective chelators [33]. The activity can be reinstated following re-exposure of the chelator-treated sections to either copper or iron salts suggesting that redox imbalance in AD is dependent on these metals. It is probable that accumulation of iron and copper is a major source of the production of reactive oxygen, which are in turn responsible not only for the numerous oxidative stress markers that appear on NFT and senile plaques, but also for the more global oxidative stress parameters observed in AD.

4. Genetics and oxidative stress

A number of mechanisms have been suggested for Aβ-associated neurotoxicity [21,34,35], including membrane depolarization [36], increased sensitivity to excitotoxins [37], and alterations in calcium homeostasis [38]. However, the influences of Aβ and other genetic factors on the pathogenesis of AD may be mediated through their effects on oxidative homeostasis. As discussed, in vivo Aβ appears to play a protective role. However, like all antioxidants, under certain conditions, Aβ also has pro-oxidant abilities. Indeed, in vitro neuronal damage by Aβ seems to be a direct result of free radicals, such that the effect can be attenuated by application of antioxidants like vitamin E [39,40] and catalase [41,42].

Mutations in the human presenilin genes 1 and 2 [43,44] are genetic factors linked to early onset of AD. Although their pathogenic mechanism are not fully understood at this point, a role for oxidative stress has been suggested. Increased presenilin 2 expression increases DNA fragmentation and produces apoptotic changes [45], which are both important consequences of oxidative damage. Apolipoprotein E is a protein that has been found to confer increased susceptibility when the ApoE4 allele is present. ApoE has been shown to be adducted with the highly reactive lipid peroxidation product, hydroxynonenal, in AD brains and cerebrospinal fluid [46]. Furthermore, ApoE is a strong chelator of copper and iron, both of which are important redox-active transition metals [47]. Another suggested genetic risk factor, bleomycin hydrolase genotype, is also associated with alterations in redox homeostasis [48].

5. Conclusion

The fact that oxidative stress plays an important role in AD pathogenesis seems clear given the evidence that research has recently provided. Markers of oxidative damage include the increase of HO-1 and 8OHG in AD brain as compared with controls. In addition, the hallmark structures of AD, NFT and senile plaques, are altered in ways characteristic of oxidative damage including AGE-modification, protein crosslinking and carbonyl- and acyl-modification. Although the source of the shift in oxidative homeostasis is still unclear, current evidence points to the fact that changes in the balance of redox transition metals, especially iron and copper, are key in the process. Both Fe and Cu are present at significantly elevated levels in AD neuropil, and detection of redox activity in AD brain can be attenuated by chelators of these key metals. It has also been demonstrated that many of the proteins that are important in their regulation, including ferritin and ceruloplasmin, show altered expression in AD and other neurodegenerative disorders. These changes could, in part, be responsible for the oxidative imbalance or may represent an attempted antioxidant response by affected cells. Taken as a whole, this research assigns a significant role to oxidative stress in AD.

However, the key aspect of the degree of cognitive decline in neurodegenerative disease is selective neuronal death. Our findings that neurons displaying protein damage also show an oxidative stress response is still in need of a mechanism that links damage and death. Determining the relative contribution of sugar- or lipid-adduction reactions, as well as direct side-chain oxidation, to the properties of abnormal inclusions will provide insight into the mechanism linking damage and death. This in turn may allow for the development of rational therapeutic protocols for specific neurodegenerative diseases, e.g. using water- versus lipid-soluble antioxidants [49] or free-radical versus carbonyl scavengers. We are encouraged by the preliminary epidemiological and

clinical studies suggesting that inhibitors of oxidative stress and glycation are effective in reducing the clinical manifestation of neurodegenerative diseases [50,51].

In recent years, there has been an increased awareness of the seminal role that both oxidative stress and redox-active transition metals play in AD as well as other neurodegenerative diseases. The stage is now set to critically examine the importance of these basic research findings as they are translated into therapeutic modalities, such as the clinical use of antioxidants and chelating agents. The next 2–3 years will be truly fascinating to watch unravel.

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