Amyloid β-peptide-associated Free Radical Oxidative Stress and Alzheimer’s Disease

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Amyloid β-peptide (Aβ) is the principal constituent in senile plaques (SP) in the Alzheimer’s disease (AD) brain. A central role for Aβ in AD has been proposed [1], based on the following observations: certain genetic mutations in the amyloid precursor protein (APP), encoded on chromosome 21, lead to familial AD; essentially all Down’s syndrome patients with chromosome 21 involvement eventually develop AD if they live sufficiently long; mutations in presenilin genes encoded on chromosomes 14 and 1, thought to be involved in APP processing and which lead to increased Aβ deposition, result in early-onset familial AD; and transgenic APP-over-expressing mice exhibit many, although not all, of the pathological characteristics of the AD brain, including Aβ deposition.

One of the most enigmatic aspects of AD research is the large number of reports of alterations in different enzymes, transport proteins, lipids, etc., in the AD brain. How can one disease have so many different alterations? This question led our group to consider the possibility that Aβ itself may be associated with free radical oxidative stress. If Aβ-associated free radical oxidative stress occurred, then neuronal membrane lipid peroxidation and protein oxidation would ensue, with subsequently altered function of membrane transport proteins and other moieties. In particular, if the Na+/K+-ATPase were inhibited by Aβ-associated free radicals or their byproducts, then the cell potential would be compromised. This would have the effect of opening voltage-gated Ca²⁺ channels, leading to large influx of intracellular Ca²⁺. The latter could also cause the release of intracellularly-stored Ca²⁺. If the Ca²⁺-ATPase were also inhibited by the Aβ-associated free radicals or their byproducts, then the excess intracellular Ca²⁺ would not be removed from the neuron. Activation of Ca²⁺-dependent degradative pathways, including proteases, endonucleases or apoptotic processes, would lead to neuronal death (Figure 1).
Figure 1. Amyloid β-peptide-associated oxidative stress model for neuronal death in Alzheimer's disease.

The electron paramagnetic resonance (EPR) technique of spin trapping involves a non-paramagnetic nitrene, which, in the presence of a transient free radical, forms a stable, paramagnetic nitroxide that is detectable by EPR. Aβ(1–42), Aβ(1–40), or Aβ(25–35), when mixed with the spin trap, N-tert-butyl-α-phenylnitrene (PBN) in metal-chelating buffers, yields an EPR spectrum [2, 3], indicating that free radicals are associated with the peptide. The free radical formed is oxygen-dependent and, based on several different studies, may be peroxy in nature (reviewed in [4, 5]). Should these processes occur in the AD brain, then alteration of many different enzymes, transport proteins, lipids, etc., in this disorder could be rationalized as discrete manifestations of the interactions of these moieties with Aβ-associated free radicals [4, 5] and the above-mentioned enigma might be better understood.
Aβ-ASSOCIATED FREE RADICAL OXIDATIVE STRESS AND AD

 Recently, many laboratories have provided considerable evidence for oxidative stress in AD [6–9]. Among other alterations, AD brain regions rich in SP show evidence of protein oxidation, while the SP-poor cerebellum does not [6]. Lipid peroxidation also is observed in the AD brain [7]. Our group and many others have shown that Aβ, in ways inhibitable by free radical antioxidants, leads to lipid peroxidation [10–14] and protein oxidation [15–17] in various brain membrane systems; generates reactive oxygen species (ROS) [15, 16]; inhibits hippocampal neuronal and cortical synaptosomal membrane ion-motive ATPases, including Na⁺/K⁺-ATPase and Ca²⁺-ATPase [18]; blocks glutamate uptake [16, 19, 20]; inhibits the activity of glutamine synthetase [2, 21, 22] (both the latter Aβ-induced alterations have the effect of increasing excitotoxic glutamate levels); causes intracellular Ca²⁺ levels to increase dramatically [15, 16, 23]; and leads to neurotoxicity in hippocampal neuronal or astrocytic cultures (reviewed in [5, 8]).

 One way in which Aβ-associated free radical oxidative stress is manifested is by membrane lipid peroxidation [10–14]. Vitamin E protects against this peroxidation, expected for a free radical process [4, 5, 11]. The chief lipid peroxidation product, 4-hydroxy-2-trans-nonenal (4-HNE), rapidly modifies proteins through Michael addition of cysteine, lysine or histidine residues [24, 25]. Exposure of Aβ to cultured hippocampal neurons produces 4-HNE at a level of 5–10 μM [26]. This alkenal, like Aβ itself [18], inhibited ion-motive ATPases, led to increased intracellular Ca²⁺ and caused cell death in neuronal cultures and synaptosomes [26]. EPR studies, in conjunction with protein-specific spin labels, showed that 4-HNE, even at 1 μM, caused a significant alteration in the conformation of cortical synaptosomal membrane proteins [27], probably accounting for altered function of these membranes exposed to 4-HNE [28]. Glutathione, known to protect neurons from 4-HNE-induced toxicity [24, 26], prevented the conformational alterations in cortical synaptosomal membrane proteins by 4-HNE [27].

 Aβ-associated free radical oxidative stress is prevented by various free radical antioxidants (reviewed in [4, 5]). Although there are undoubtedly other sources of oxidative stress in the AD brain, including, among others, activated microglia, transition metals and advanced glycation endproducts (reviewed in [8]), given the importance of Aβ in AD, free radical oxidative stress associated with this peptide most certainly plays an important role in neuronal death in the AD brain. In addition to direct oxidation of neuronal lipids and proteins or their modification by the lipid peroxidation product HNE, Aβ may also lead to neuronal death in AD by other mechanisms, e.g. induction of detrimental transcription factors leading to production of harmful proteins and apoptosis, inflammatory responses from microglia, or altered mitochondrial function. The common factor in all these processes is oxidative stress, and this suggests that brain-accessible free radical scavengers should be considered for potential therapeutic intervention/prophylaxis in AD. Initial reports using this strategy, consistent with our model for AD neuronal death (Figure 1), are encouraging [29].
In our laboratory, research continues on mechanistic investigations of Aβ-associated free radical oxidative stress. Methionine residue 35 appears to be involved since: Aβ(25–35) incubation leads to free radicals and formation of methionine sulfoxide; Aβ(25–35)-Met-SO, with the sulfoxide already synthesized in the peptide, yields no EPR signal, neither is this peptide toxic to the oxidatively-sensitive enzyme glutamine synthetascape; substitution of methionine by norleucine in Aβ(25–35) (of similar length and hydrophobicity as Met, but with no sulfur), yields no EPR spectrum and is not toxic to GS; and Aβ(25–34), in which Met is absent, yields no EPR spectrum and is not toxic to GS. These negative results both point to the importance of Met-35 and suggest that metal-catalyzed processes are probably minimally involved, otherwise, one would have predicted similar reactions in Met-modified peptides, as in Aβ itself. Additional research on mechanisms of Aβ-associated free radicals, the membrane alterations that are induced by Aβ and its chief lipid peroxidation product 4-HNE, the relevance of Aβ-associated free radical oxidative stress to neurotoxicity in Alzheimer’s brain, and potential pharmacological strategies to inhibit these processes [30], are in progress.

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