Chapter 2
Oxidative Stress and Alzheimer's Disease

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Abstract Oxidative stress is the inevitable result of life's requirement to reduce molecular oxygen to water for cellular respiration and energy metabolism. For a number of reasons, the human brain appears particularly vulnerable to oxidative stress, which has necessitated elaboration of complex antioxidant defenses in order to maintain oxidative balance. With advanced age, oxidative balance wanes in favor of oxidative stress, which sometimes results in disease, in particularly age associated sporadic or environmentally driven diseases such as Alzheimer's disease, cardiovascular disease, and cancer. Over the last 20 years, our laboratory has investigated oxidative stress by numerous in situ techniques and have identified oxidative stress-associated adducts, redox active transition metals, and metal associated proteins, not only within pathological lesions of the AD brain, but also unaffected brain prior to the onset of overt structural pathology. We have further demonstrated that oxidative stress decreases with increasing pathology, especially amyloid, suggesting that hallmark lesions in AD are more likely a productive response than a deleterious event. These and other findings continue to indicate the need to examine oxidative stress in greater detail, as well as expand the universe of antioxidant therapies, particularly as classical lesion-based therapies continue to fail.

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2.1 Oxidative Stress

The reduction of molecular oxygen to water during the process of cellular respiration drives both the ATP synthesis necessary to sustain life, as well as the production of free radicals which can potentially destroy life [1]. The latter no doubt precipitated the evolution of elaborate antioxidant defenses, such that the pro-oxidant tendency of molecular oxygen in the intact organisms is balanced somewhere near the organism's ability to neutralize free radicals with near 100 % efficiency. Such is the nature of life for much of the lifespan of the organism.

Commensurate with the aging process, however, is a slow diminution in antioxidant defenses, and the "slow burn" of oxidative stress as a gradual but steady destructive force. Among the antioxidant mechanisms are vitamins A, C, and E, glutathione, and a number of enzymes that facilitate electron transfer to a nontoxic species, such as catalase, superoxide dismutase, and glutathione peroxidase, and each has been shown to decrease with age. It is therefore not surprising that virtually all sporadic or environmentally driven chronic diseases (cancer, cardiovascular disease, neurodegeneration) are both age-related and associated with oxidative stress [2].

The brain appears to be vulnerable to oxidative stress via a number of mechanisms. Glutathione and vitamin E have been shown to be in limited supply in the brain, in the face of an inherently high metabolic activity, dominated by oxidative metabolism [3]. Iron content is increased in some brain regions (e.g., substantia nigra), which is a potent source of free radicals via the Fenton reaction (ferrous iron plus hydrogen peroxide yielding ferric iron and an hydroxyl radical, the latter being a potent, nonselective oxidant species).

2.2 Involvement of Oxidative Stress in Alzheimer's Disease

Heme oxygenase. Once the steady state is breached in favor of oxidative stress, the deleterious effects are far-reaching. Indeed, every category of biomacromolecule, e.g., carbohydrates, protein, lipid, nucleic acids, is a potential target [2]. Among the earliest in vivo studies indicating the presence of oxidative stress in brain tissue was the pioneering study by Mark Smith in the 1990s on the role of heme oxygenase in the AD brain [4]. Heme oxygenase is an endogenous antioxidant that exists in multiple isoforms. Most notable is Heme oxygenase-1 (HO-1), which is inducible in the presence of oxidative stress, and processes heme to biliverdin, while generating redox-active iron and carbon monoxide. Carbon monoxide then may have a neurotransmitter function, and it is further of note that recent studies have shown a neuroprotective effect of carbon monoxide. Heme oxygenase was shown to be markedly upregulated in the AD brain within lesional as well as unaffected tissue, which laid the groundwork for subsequent studies and an expansion of our knowledge of oxidative stress in human brain in vivo, prior to which oxidative stress was only studied in highly artificial experimental constructs.

Advanced glycation and lipid peroxidation. In previous studies, we have since shown that protein adducts pentosidine and pyralline, formed via the Maillard reaction and advanced glycation, a process accelerated by molecular oxygen, are abundant in AD brains, not only within pathological lesions, but within pre-pathological vulnerable neurons [5]. We have also demonstrated lipid peroxidation end products hydroxynonenal and malondialdehyde in AD, both within lesions and within pathologically normal tissue. Both sets of adducts, advanced glycation end products and advanced lipid peroxidation end products, lead to the formation of intramolecular and intermolecular cross-links which render otherwise soluble proteins insoluble and resistant to degradation [6]. Carboxymethyllysine, an adduct associated both with advanced glycation and lipid peroxidation, has similarly been shown by us to be intimately associated with the AD brain [7]. In a recent study, we have also shown that hydroxynonenal in particular may accumulate less within lesions as they do within neurofilaments of axons [8]. The lysine-rich nature of neurofilaments, and lysine-lysine adducts may therefore contribute to the aging and disease process by disrupted slow axonal transport.

Nitration. In another study, we examined the brain for nitration of tyrosine residues, as peroxynitrite is a source of hydroxyl radical-like reactivity, and it directly oxidizes proteins and other macromolecules with resultant carbonyl formation from side-chain and peptide-bond cleavage [9]. Indeed, we found increased protein nitration in neurons, including but not restricted to neurofibrillary pathology, while control brains showed no such involvement. These data not only supported oxidative damage in AD, but that it was not necessarily limited to poorly soluble fibrils such as neurofibrillary tangles, but much like other indicators of oxidative stress such as AGE and lipid peroxidation adducts, reflects a broad and early event.

Damage to nucleic acids. DNA modification intuitively speaking appears more relevant to cancer biology than neurodegeneration, given the importance of acquired genetic alterations in cancer and the fact that the vast majority of neurons, including vulnerable neurons in AD, are post mitotic. Nevertheless, 8-hydroxyguanosine modification has been shown to be increased in AD brain tissue. Additionally, and perhaps more importantly, oxidation of RNA has been linked to AD in recent studies. There is of interest given the single stranded nature of RNA. It may be that oxidative stress induced damage to RNA results in sublethal cellular injury, altering protein translation, and disrupted cellular metabolism in favor of neurodegeneration [10].

Heavy metals. Transition metals, and in particular copper, iron, and zinc, are potent catalysts of free radicals [11–14]. Evidence suggests that metals may accumulate in the brain with age and disease, accelerating oxidative damage and possibly contributing to amyloidosis [15]. Using a modification of the Prussian blue reaction to select for redox state, we have demonstrated specifically redox-active iron in the AD as well as Parkinson disease. Moreover, the accumulation within lesions suggests that insoluble proteinaceous accumulations may be a "sink" for free radicals within the brain, suggesting that lesions themselves are more in line with Darwinian theory, or adaptation to the environment, than a priori indicators of toxicity [3, 16–18].

Are hallmark lesions a response to oxidative stress? The selective nature of the neurodegenerative process in the AD brain is difficult to explain in the face of a relatively selective disease process, affecting certain brain regions and certain neuronal subsets during the course of the disease. Whether the overall level of free radical production differs in vulnerable areas, or whether antioxidant defenses are deficient in those same areas for one reason or another, is difficult to discern precisely, although evidence for both mechanisms has been offered in the literature [19, 20]. It may further be worth noting that the major pathogenic hypothesis, or the putative amyloid cascade, has shown little region selectivity aside from "cortex" and is in fact less selective than phospho-tau accumulation, which is manifestly a downstream event [16]. It is also of some interest that oxidative stress precedes all types of pathological lesions, and that in one study, the magnitude of oxidative stress *decreased* with increasing amyloid, suggestive of a neuroprotective effect of amyloid production [21]. Clinical trials investigating the efficacy of antioxidant therapy in AD have been disappointing (although no less disappointing that all of the trials targeting amyloid-β (Aβ)), and it may be that the complexity of redox biology and approaching and altering brain chemistry in this regard, exceeds both understanding and therapeutic strategies available to date. Nevertheless, as the scientific community comes to realize more and more that lesion targeting is based on rare Mendelian amyloidosis and not strict age-related sporadic conditions, continued study of oxidative stress in vitro, in vivo, and as a therapeutic approach, is certainly warranted.

2.3 Conclusion

Oxidative stress is intimately intertwined with chronic, age-related disease processes, including AD. The specific vulnerability of the brain to oxidative stress may in part explain the abundance of evidence for its existence within both lesional and non-lesion tissue. Moreover, the existence of specific adducts of oxidative stress has, since the 1990s facilitated the production of antisera against those adducts, which has in turn allowed in-situ examination for oxidative stress hallmarks in humans, rather than through highly artificial experimental constructs prior to this time. The data have generally furthered the evidence for oxidative stress and provided a temporal dynamic that precedes traditional hallmark lesions. As lesion targeting continues to fail, a more substantial investment in oxidative biology as it pertains to AD is warranted.

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