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Review

Metal dyshomeostasis and oxidative stress in Alzheimer's disease

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ABSTRACT

Alzheimer's disease is the leading cause of dementia in the elderly and is defined by two pathological hallmarks; the accumulation of aggregated amyloid beta and excessively phosphorylated Tau proteins. The etiology of Alzheimer's disease progression is still debated, however, increased oxidative stress is an early and sustained event that underlies much of the neurotoxicity and consequent neuronal loss. Amyloid beta is a metal binding protein and copper, zinc and iron promote amyloid beta oligomer formation. Additionally, copper and iron are redox active and can generate reactive oxygen species via Fenton (and Fenton-like chemistry) and the Haber–Weiss reaction. Copper, zinc and iron are naturally abundant in the brain but Alzheimer's disease brain contains elevated concentrations of these metals in areas of amyloid plaque pathology. Amyloid beta can become pro-oxidant and when complexed to copper or iron it can generate hydrogen peroxide. Accumulating evidence suggests that copper, zinc, and iron homeostasis may become perturbed in Alzheimer's disease and could underlie an increased oxidative stress burden. In this review we discuss oxidative/nitrosative stress in Alzheimer's disease with a focus on the role that metals play in this process. Recent studies have started to elucidate molecular links with oxidative/nitrosative stress and Alzheimer's disease. Finally, we discuss metal binding compounds that are designed to cross the blood brain barrier and restore metal homeostasis as potential Alzheimer's disease therapeutics.

1. Introduction

Copper, zinc and iron are the three most abundant biometals in mammals, and play catalytic and structural roles in many enzymatic processes. The redox potential of copper and iron are what facilitate their catalytic activity. However, this potential can also cause oxidative stress mainly via Fenton (and Fenton-like) chemistry (Jomova and Valko, 2011; Stohs and Bagchi, 1995). Conversely, zinc is redox inert and usually plays a structural role in protein folding and stability and coordinating protein–protein interactions (Eide, 2011). Zinc also has unique antioxidant properties that enable it to modulate oxidative stress via protection of protein sulfhydryl groups (Powell, 2000). Regulation of copper, zinc and iron are intimately linked at both an organismal and cellular level and metal dyshomeostasis is featured in numerous human diseases. Much of the current knowledge about regulation of copper, zinc and iron has come from our understanding of genetic disorders such as Menkes and Wilson diseases (Wang et al., 2011), acrodermatitis enteropathica (Andrews, 2008) and aceruloplasminemia (Vassiliev et al., 2005). However, in the past decade there has been considerable interest in the metal dyshomeostasis that is a feature of several neurodegenerative conditions. In the case of Alzheimer's disease (AD) there are disturbances in the levels of copper, zinc and iron in localized regions of the brain associated with disease pathology. Although the interaction of extraneuronal copper, zinc and iron with cytotoxic amyloid beta (Aβ) had been known for 15–20 years, in the last few years much progress has been made in understanding the associated cellular changes to metal-protein trafficking and signaling cascades. Further elucidation of these pathways will not only help to understand what role metals play in oxidative stress, a feature of AD, but also rationalize development of potential therapeutic compounds targeted to restore metal balance in the brain. Several such compounds, in particular 8-hydroxyquinolines, have shown some efficacy in both animal and human trials and will be discussed in this review.

2. Alzheimer's disease

AD is a progressive neurodegenerative disorder that involves progressive cortical and hippocampal neuron loss and corresponding cognitive decline. A complex interaction of multiple genetic and environmental risk factors is thought to underlie the etiology of AD but age is still considered the number one risk factor for sporadic cases, which make up >95% of all cases. The remaining cases

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have a clear genetic inheritance. Termed familial Alzheimer's disease (FAD) they are caused by mutations in the *amyloid precursor protein* gene (*APP*), *presenilin 1* (*PSEN1*) or *presenilin 2* (*PSEN2*) respectively (Bertram et al., 2010). Two pathological hallmarks of AD are aggregation of Aβ, the major constituent of extraneuronal senile plaques, and hyper-phosphorylation of Tau, which forms intracellular neurofibrillary tangles (NFTs). Aβ peptides are released from APP by the sequential protease activity of β-secretase (BACE1) (Cole and Vassar, 2008) and γ-secretase, a multiprotein complex that contains either PSEN1 or PSEN2 as its catalytic constituent (Steiner et al., 2008). The length of naturally occurring Aβ can vary but is typically a peptide of 39–42 amino acids. Of the 160 + mutations that have been identified in FAD cases the majority cause an increase in the prevalence of a longer form; $A\beta_{42}$, which is considered particularly neurotoxic and more prone to self-aggregation than shorter peptides. The ratio of $A\beta_{42}$ to $A\beta_{40}$, is considered a surrogate plasma biomarker and may be a useful early predictor of AD disease progression (Koyama et al., 2012).

Senile plaques are composed primarily of extraneuronal aggregated Aβ but also contain relatively high amounts of Cu and Zn, which are known to precipitate Aβ *in vitro* (Bush et al., 1994a,c). Many researchers now believe that soluble oligomers are the most toxic form of Aβ and that these may start to form early in AD disease progression, long before fibrillary and plaque pathology becomes evident (Larson and Lesne, 2012). Alarmingly, PET imaging of Aβ, combined with cerebrospinal fluid (CSF) measurements of Aβ and Tau, suggest that AD may start some decades before it becomes symptomatic and plaques begin to accumulate (Weiner et al., 2012). Indeed, plaques may actually be a last ditch cellular attempt to "wall off" potentially toxic Aβ oligomers. There is also considerable evidence to indicate perturbations to Aβ-degradation pathways are associated with AD (Wang et al., 2006; Kurz and Perneczky, 2011). Therefore, an imbalance between Aβ production and Aβ clearance is likely to underlie the disease process. Tau plays an important physiological role in cytoskeletal stability and axonal transport in neurons as it controls microtubule assembly. Perturbed microtubule formation and accumulation of phosphorylated tau in NFTs is a contributing factor to neuronal dysfunction and cell death in AD (Lovestone and Reynolds, 1997). There is substantial evidence to demonstrate that Aβ and Tau may act synergistically to potentiate neuronal dysfunction in AD (Huang and Jiang, 2009). Furthermore, molecular evidence suggests tau hyperphosphorylation is the result of perturbed cellular signaling cascades that may, to some extent, be mediated by Aβ (Hernandez et al., 2010).

3. Metal dyshomeostasis and AD

An imbalance of metal homeostasis in the brain is thought to play an important role in the pathogenesis of AD (Bush, 2012). The mammalian brain contains an intrinsically high concentration of Cu, Zn and Fe ions compared to other tissues, which is reflective of its high requirement for these metals in numerous metal-dependent enzyme and metabolic processes (Popescu and Nichol, 2011). The concentrations of Cu, Zn and Fe in the brain are tightly regulated at the level of the blood brain barrier (BBB) (Bobilya et al., 2008; Bradbury, 1997; Zheng and Monnot, 2012). In AD brains large net increases in Cu, Zn and Fe have been reported compared to healthy age-matched controls – Cu: 390 μM vs. 70 μM, Zn: 1055 vs. 350 μM, Fe: 940 μM vs. 340 μM – respectively (Lovell et al., 1998). It must be noted that there is some debate in the literature regarding these values. In particular, Schrag and colleagues performed a meta-analysis of the existing literature and concluded that total brain Fe and Cu levels are not significantly altered in AD brain vs. age-matched controls (Schrag et al., 2011). Despite this, numerous methods including proton induced X-ray emission, immunohistochemistry and synchrotron X-ray fluorescence have detected focalized concentrations of Cu, Zn and Fe in areas of amyloid plaque pathology from AD patients and transgenic mouse models (Danscher et al., 1997; Friedlich et al., 2004; Lee et al., 1999, 2002; Lovell et al., 1998; Miller et al., 2006; Stoltenberg et al., 2007; Suh et al., 2000). Systemic metal dyshomeostasis is also evident in AD patients with higher than age-matched normal levels of Cu reported in both the CSF and serum respectively (Basun et al., 1991; Squitti et al., 2002). In contrast, plasma Zn levels normally decline with age (Bunker et al., 1987; Monget et al., 1996; Munro et al., 1987; Ravaglia et al., 2000) but there is evidence that both plasma and CSF Zn levels are further depleted in AD patients compared to age-matched control patients (Basun et al., 1991; Baum et al., 2010; Molina et al., 1998). Hence, global changes to Cu and Zn are a feature of AD.

4. Oxidative stress and AD

4.1. Markers of oxidative stress

Increased oxidative stress is associated with normal aging but it is further exacerbated in several neurodegenerative disorders including AD and Parkinson's disease (Jomova et al., 2010). Mounting evidence implicates oxidative stress as an early event in AD disease progression mediated, in part, by pathological increases in Aβ:metal interactions that facilitate reactive oxygen species (ROS) production. Markers of oxidative stress that are elevated in AD brain include lipid peroxidation, DNA oxidation, protein oxidation, advanced glycation end-products (AGEs) and reactive nitrogen species (Butterfield et al., 2011). Homocysteine (Hcy) is a potential blood biomarker of AD as numerous groups have reported elevated serum or plasma Hcy levels in AD patients compared to age-matched controls (Clarke et al., 1998; Doecke et al., 2012; Gallucci et al., 2004; Guidi et al., 2005; Joosten et al., 1997; McCaddon et al., 1998; Quadri et al., 2004; Selley, 2003; Trojanowski et al., 2010). The mechanism of Hcy induced toxicity has not been fully elucidated but strong interactions with Fe (Baggott and Tamura, 2007) and Cu (White et al., 2001), together with associated increases in protein carbonyls suggest a causal relationship between Hcy and oxidative damage (Sibrian-Vazquez et al., 2010). However, circulating Hcy increases normally with age and there is some doubt in the literature as to whether Hcy levels are elevated in AD (Ariogul et al., 2005; Luchsinger et al., 2004; Mizrahi et al., 2003). The generation of Hcy is part of the complex and synergistic methionine biosynthetic pathway and is affected by factors such as dietary intake of folate and vitamin B_{12} (Cito et al., 2010). Population differences and confounding medical conditions may also account for some of the contradiction in the literature (Cito et al., 2010). Another factor that contributes to oxidative stress is a reduced ability to cope with a rise in pro-oxidants such as Aβ and there is evidence that major antioxidant defense systems are perturbed in AD (see chapter 7).

4.2. Mechanisms of oxidative stress

Redox cycling between Cu^{1+}/Cu^{2+} and Fe^{2+}/Fe^{3+} facilitates the activation of molecular oxygen, a process utilized by numerous enzymes including cytochrome-c oxidase (CCO), an integral part of the mitochondrial electron transport chain and ATP production (Yoshikawa et al., 2011). However, unregulated interaction of Cu and Fe with molecular oxygen also facilitates the generation of ROS. Levels of Cu and Fe are intrinsically high in the mitochondria, a highly oxygenated microenvironment that produces