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Review Article

Alzheimer's Pathogenesis, Metal-Mediated Redox Stress, and Potential Nanotheranostics

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Abstract

Alzheimer's disease (AD) characterized by insoluble amyloid-β (Aβ) deposits, neurofibrillary tangles (NFTs), and neuronal demise. The influence of environmental and genetic factors on AD progression remains elusive, however evidence suggests biometal dyshomeostasis elicits neuronal death, neuroinflammation, and accumulated oxidative damages in AD brain. As such, three pathways have been identified that result from abnormal biometal accumulation and increased levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in AD brain parenchyma: (1) the damage caused by direct oxidation of cellular components such as DNA and proteins; (2) the oligomerization of Aβ and NFTs, and (3) the promotion of apoptosis through NF-κB signaling pathway. Finally, given recent developments in nanotechnology, we have briefly reviewed potential nanotheranostic agents as potential AD theranostics.

Keywords: *Alzheimer's Disease; Redox Stress; Aβ Amyloid; Tau Protein; Biometals; Metal Chelators; Nanotheranostics; Blood-Brain Barrier*

Introduction

Alzheimer's disease (AD) is the most common form of senile dementia, affecting at an estimated of 5.8 million Americans, as trends are projected to reach 13.8 million due to the baby boomers [1]. Clinical significance includes irreversible memory impairments and can lead to decline of motor and sensory functions [2]. Abnormal accumulation of insoluble misfolded and post-translationally modified proteinaceous deposits, amyloid-β plaques and neurofibrillary tangles (NFTs), are most common features of AD [2]. In brief, Aβ are the principal components of senile plaques and NFTs are composed of hyperphosphorylated tau proteins. The majority of cases are classified as sporadic. However, between 5% and 10% of cases are familial and have an autosomal dominant inheritance pattern with variable penetrance; suggesting the determinants of disease are polygenic and multifactorial [3].

Aβ is generated by post-translational synergistic cleavage of amyloid precursor protein (APP) via β- and γ-secretases. Normally, APP catabolism generates Aβ isoforms with carboxyl-terminal heterogeneity of between 39 and 43 residues [4]. The Aβ1-40 form (40 amino acid residues) is the major soluble Aβ species generated in brain and is typically found at low nanomolar concentrations in cerebrospinal fluid (CSF) [5]. The Aβ1-42 form (42 residues) is generated at levels 10-fold lower than Aβ1-40. It is also more fibrillogenic and heavily enriched in the interstitial amyloid plaques. Interestingly, the ratio of soluble Aβ1-42 to Aβ1-40 is increased in familial AD. This observation and the greater propensity of Aβ1-42 to form neurotoxic oligomers has led to this isoform being considered as the key pathogenic Aβ species in AD [6].

Previous research determined insoluble Aβ deposits mediates neurodegeneration, known as the amyloid hypothesis. However, emergence of recent data hypothesized amyloid alone may not fully account for pathogenesis. Furthermore, plaque load shows weak correlation with cognitive status [7]. Experimental evidence suggests highly neurotoxic soluble oligomeric intermediates are the key pathogenic Aβ species in AD but insoluble amyloid-β deposits [8,9].

Experimental data suggest that biometals may play a key role in Aβ pathology and AD pathogenesis, and mechanisms for initiation and propagating Aβ pathology appear to be polygenic and multifactorial. For example, pathological proteins are inducted by direct interaction with Aβ or tau. It also influences the generation of ROS favoring environmental conditions that induce AD pathology [10]. Notably, oxidative brain conditions exacerbate Aβ levels causing a negative feed-forward loop [11].

Furthermore, increased metal and ROS levels may also activate the nuclear factor kappa B (NF-κB) signaling pathway. The NF-κB pathway induces neuronal apoptosis and plays a role in AD neurodegeneration [9,10,12,13]. Herein, this review will focus on the roles of biometal-mediated oxidation in AD pathology and review a model in which activation of the NF-κB signaling pathway ultimately leads to Aβ deposition. In addition, potential novel strategies to treat AD using nanotheranostics will be discussed.

Metal-mediated redox stress in Alzheimer's disease

Evidence suggests environmental metal exposure or a homeostatic imbalance (such as copper ions) are major risk factor for AD. In fact, expression of APP is modulated by copper ions [10]. Furthermore, primary cortical neurons and embryonic fibroblasts from APP null mice exhibited significantly elevated levels of copper compared to wild type mice [14,15]. Collectively, these data suggest the cross-regulatory action between APP and biometal imbalance carries profound consequences for AD pathophysiology [16].

One likely consequence of dyshomeostasis of cerebral biometals is increase redox potential of the brain parenchyma. Consistent with this model, AD brain show evidence of extensive, wide spread and long-term ROS-mediated oxidative damage and specific binding of redox-active biometals are likely to elevate non-specific oxidation of Aβ, tau, and APP [17]. Moreover, Aβ is a metallopeptide with a high affinity for copper [18] and zinc [19, 20]. Copper binding in particular has been shown to promote Aβ oligomerization and redox activities linked to AD pathology [21]. Thus, biometal dyshomeostasis may contribute to the generation of pathology via at two mechanisms by increasing interaction of these metalloproteins with oxidizing metals and through promotion of an oxidative environment in AD brain parenchyma.

Analysis of micro particle-induced x-ray emission (μ-PIXE) observed abnormal enrichment of Cu, Fe, and Zn when Aβ amyloid plaques were present a post-mortem AD brain [22]. A micro X-ray fluorescence (μ-XRF) in combination with laser capture microdissection (LCM) μ-XRF provides higher sample penetration depth (1000 μm) and spatial resolution (0.1 μm) compared to μ-PIXE (100 μm and 0.3 μm, respectively) [23]. In combination these techniques allow high resolution analysis while minimizing possible background signal from neuropils and other cellular components. Our data confirmed previous results stating amyloid plaques are enriched for Cu, Fe, and Zn. In addition, analysis revealed similar biometal profiles in submicron size amyloid plaques associated with early stages of AD amyloidosis [24]. Plaque sulfur levels were also found to be abnormally high. High sulfur signals suggest an abundance of di-sulfur bonds and S-glutathionylation, markers of oxidative conditions [25]. These data are consistent with an important role for oxidative stress and damage in plaque formation [26].

Metals promote Aβ aggregation and redox activity

Former results attested Aβ aggregation mediated by Zn and Cu through histidine residues and N-terminal region that support metal ion binding [27,28]. One study suggests that under metal-free conditions Aβ is thermodynamically soluble at physiological concentrations [29]. Metal ion binding appears to alter Aβ conformation and lower the kinetic barrier to precipitation [30].

In addition to promoting aggregation, Aβ/Cu, and to a lesser extend Aβ/Fe complexes, can engage in redox reactions. Aβ reduces Cu(II) and Fe(III), to Cu(I) and Fe(II) respectively with a concurrent generation of ROS- H_2O_2 and OH•.

The likely mechanistic reaction scheme is as follows [31]:

$$(A\beta)_2 + M^{(n+1)+} \rightarrow A\beta{:}A\beta^{+}\bullet + M^{n+}$$

The reduced Fe(II)/Cu(I) then reacts with molecular oxygen (O_2) to generate the superoxide anion (O_2^-)

$$M^{n+} + O_2 \rightarrow M^{(n+1)+} + O_2^-$$

The O_2^- generated undergoes peroxidation to H_2O_2 and O_2 either by the SOD enzyme or spontaneously.

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Similarly, the reduced metals can directly interact with H_2O_2 to generate the highly reactive OH• by the Fenton reaction (Cu(I) which catalyzes this reaction at a rate-constant magnitude higher than that for Fe(II)) [32].

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + OH\bullet + OH^-$$

Aβ/metal complexes can recruit other biomolecules to deradicalize the Aβ peptide component and regenerate the complex for further redox reactions and ROS generation [33-35]. The hydroxide radicals generated by these reactions have been implicated in the cytotoxic actions of Aβ [36].

Aβ peptides exposed to exogenous ROS oligomerize and form aggregates [37]. Conversely, oligomerization is attenuated by catalase and ROS quenching metals [38]. Thus, the metal-mediated redox activity of Aβ may generate ROS that promote the peptides own modification and pathological oligomerization [39-41]. Data on Aβ/metal complexes comes mostly from *in vitro* experiments [42]. However, taken together these findings are compelling evidence for direct Aβ/metal redox activity as a major source of the widespread neurodegeneration and oxidation damage that occurs with AD [26, 43-45].

The 5'-untranslated region (5'UTR) of APP mRNA has a functional iron-response element (IRE) [46] that is consistent with a role for APP as a redox-active metalloprotein [47]. We found redox-active Fe^{3+} and Cu^{2+} but not Zn^{2+} ions promoted APP expression via its 5'UTR in a dose-dependent manner [48].

Metal dysregulation and chelation strategies

A plethora of evidence including meta-analysis, *in vitro* and *in vivo* and pre-clinical studies align with the notion that biometal imbalance is one of the risk factors for AD. As such, the metal hypothesis stresses the implication of dysfunctional endogenous mechanisms that lead to toxicity build up [49,50]. For example, microarray expression profiling the dichotomy between AD and healthy brains found a genetic dysregulation [51]. Compared to age-matched controls, expression levels for metal regulatory genes such as metallothionein III (MT-III) and metal regulatory factor-1 (MTF-1) are decreased 4-fold in AD brain [52]. Moreover, protein levels of MT-III are also attenuated in AD brain [53,54]. As such chelation therapies may benefit AD patients as a therapeutic [55,56].

Metal chelators dissolve amyloid deposits in post-mortem AD brain and attenuate cerebral Aβ load in an AD transgenic mouse model [57,58]. In fact, administration of the metal chelators desferrioxamine (DFO) or clioquinol (CQ) has been reported to be efficacious for AD patients [59-61]. Intramuscular DFO reduced cognitive decline over two years [59,62]. Furthermore developed the novel amyloid-targeting metal chelator XH1 that also shows promise as a therapeutic agent for AD [63]. Strategies to attenuate side effects is through the production of novel low-affinity metal-complexing agents that show higher specificity for particular transition metals called metal-

protein attenuating compounds (MPAC). One compound is PBT2 which blocks the binding of Cu (II) and Zn (II) to proteins and inhibits Aβ biogenesis [64]. PBT2 reduced β-amyloid load in a transgenic mouse model of AD. Most recently, clinical trials of PBT2 significantly and specifically reduced Aβ levels in the CSF of patients with minimal adverse effects [65-67]. The issue of toxicity is critical in drug development. Findings for PBT2 suggest the MPAC strategy is likely to minimize undesirable side effects of chelating therapies while maintaining efficacy in treating AD [65]. Further advances under development for MPAC include combining these agents with nanoparticle delivery systems. Furthermore, elucidating access in to the CNS is important for strategies pathways due to the hydrophilicity with chelating agents. High metal affinity and inability to distinguish between transition metals can lead to neurotoxicity. Therefore, nanoparticles have been developed in mitigating these issues.

Nanoparticle drug delivery systems and nanotheranostics

Restrictive passage imposed by the blood-brain barrier (BBB) is the major impediment for efficient cerebral drug delivery [51]. As the brain's main defense mechanism against foreign pathogens and toxins, the BBB is complex and highly regulated [68]. The fenestrated, though extremely tightly packed, endothelial cells of the BBB prohibit the entrance of drugs and large particles to the brain by regulating transport mechanisms at the cell surface [11]. Efforts to improve the efficacy and limit the adverse effects have been sought though the use of nanotechnology as a promising avenue [69].

For example, dendrimers are large, spherical, branched molecules. Interestingly, low concentrations of dendrimers inhibit aggregation of the Aβ peptides and the prion protein (PrP185-208) forms amyloid in spongiform encephalopathies (TSEs) [70]. Administration of hydrated fullerene (carbon nanosphere) can also inhibit Aβ fibrillation and improved cognitive performance in a rodent AD model [71]. The mechanism for these molecules anti-amyloid activity appears to involve suppression of peptide nucleation, an early step common to many amyloidosis pathways. As therapeutic anti-amyloid drugs dendrimers and fullerenes have the additional advantage of showing relatively high BBB permeability.

Other efforts to penetrate the BBB is through imitating low-density lipoproteins (LDL) facilitate passage across the BBB [72] since they can be taken up without producing environmental changes or disrupting the integrity of the barrier [73,74].

Experiments have demonstrated that nanoparticles of the rapidly biodegradable polybutylcyanoacrylate (PBCA) can be successfully delivered to the brain of living rats [75]. Moreover, PBCA nanoparticles have been used to increase tacrine levels in the brain through intravenous methods [76]. In brief, the researchers concluded tacrine concentration was enhanced by 4.07-fold. The same group applied PBCA to enhance delivery of rivastigmine by coating it with PBCA and observed an increase of 3.82-fold compared to a free drug.

Chelation therapy has been investigated to solubilize Aβ plaques. An application of a quinoline derivative, clioquinol (CQ) in a transgenic mouse model of AD resulted in a 49% decrease in Aβ deposition as compared controls [57]. Upon production of PBCA-CQ NPs, results produced a more efficient brain entry and a reduction of aggregated Aβ in double transgenic mice (APP/PS1) [77].

Nanoparticles are also promising tools for *in vivo* imaging and diagnosis of amyloidosis. Thus, imaging and contrast agents for amyloid have been developed. Assays for AD biomarkers such as Bio-barcode assay is an innovative technique that uses nanoparticles to measure levels of soluble Aβ oligomers (ADDLs) in biological fluids. The Bio-barcode assay can measure ADDLS in human CSF, which is beyond the sensitivity of conventional techniques [78]. Furthermore, to detect and remove plaques Siegemund and his team harnessed the dye, Thioflavin-T (ThT). Although ThT is hydrophilic, the dye was encapsulated within a PBCA shell composed of a polystyrene core given ThT the ability to target and identify Aβ fibers with the onset of the disease in transgenic mice using confocal laser scanning microscopy [79]. Overall, the inherent power of nanofabrication to generate materials with precise physiochemical properties also suggests that there is a good possibility of overcoming current and future limitations of nanotheranostics.

Reactive oxygen/nitrogen species and Alzheimer's disease

The role of nitric oxide (NO) in AD pathophysiology has not been elucidated. Reports show contrasting results of either neurotoxicity or neuroprotective actions [80-82]. Study's suggests the neurotoxic mechanism of NO interacts with superoxide (O_2^-) generating peroxynitrite ($ONOO^-$) causing a downstream cascade of elevated of reactive nitrogen species (RNS) [83-85], nitroxidative stress [85,86] and induction of apoptosis in neuronal cells [87,88]. Conversely, neuronal expression of NO has been observed to be protective under conditions of ischemia reperfusion [89,90] through inhibition of inositol-1,2,5 triphosphate and leukocyte accumulation [91,92]. Thus, strategies to administer NO as a treatment for AD are being considered [93]. Overall, biometal homeostasis is critical to ROS generation in cerebral tissue. Thus, restoring dysregulation of metals in AD is likely to be key for ensuring NO plays a helpful neuroprotective role as opposed to contributing to pathology.

Increased metal level has been prone to elevate ROS which leads to the transition of insoluble tau filaments through the association with cerebral dysregulation. Increase of NTF depositions leads to oligomerization and eventual neurodegeneration [94-96]. Secondly, elevated ROS have been associated with compromising plasma membrane integrity and release of oxidized fatty acids into the extracellular space causing more production of tau filament polymerization [97]. Thirdly, ROS has been observed to inactivate enzymes such as isomerase Pin1 which mediates tau dephosphorylation. Consistent with this finding, Pin1 has been shown to be oxidized and down-regulated by conditions in AD hippocampus [98].

NF-κB and Alzheimer's disease

NF-κB plays a major role in the activation of inflammatory responses and is critical in oxidative stress and neuronal apoptosis mechanisms [99]. Importantly, NF-κB is activated by RNS. Increase of RNS levels have been observed with redox-active metals. Thus, NF-κB may be a useful downstream target for ameliorating metal-mediated oxidative stress and associated neurodegenerative pathologies. Consistent with this therapeutic strategy, administration of the NF-κB agonist indomethacin dramatically reduced β-amyloid load in a transgenic mouse model of AD [100,101]. NF-κB levels also appear to be abnormally high in the brains of AD patients [102]. NF-κB inhibitor such as transportan10 has been reported to mediate a protective role against ROST generation in cultured glial cells [103]. This suggests NF-κB may also have efficacy in directly attenuating Aβ/metal mediated oxidative stress. As such, NF-κB antagonists are promising agents for ameliorating the neurodegenerative effects of metal-induced oxidative stress. However, recent findings suggest a greater understanding of the complex pathways mediated by NF-κB is needed before considering these agents for clinical trial. Administration of high doses of hypericin, a transient activator of NF-κB, has been reported to induce apoptosis. However, surprisingly, at low doses hypericin appears to protect against Aβ cytotoxicity [104]. Similarly, at least one class of NF-κB inhibitor activates caspase3 and caspase6, proteases associated with apoptosis [42,105].

NF-κB, in common with NO, appears to be either protective or cytotoxic to neuronal tissues depending on conditions in the surrounding brain parenchyma. Unfortunately, the critical conditions that differentiates NF-κB as a pro-apoptotic agent from an anti-apoptotic agent remain to be characterized. It is to be hoped the elucidating the pathways mediated by this promising drug target will become a focus of effort in the near future.

Conclusion

The vast majority of therapeutic strategies over the last 25 years have focused on reducing levels of the Aβ peptide in brain. However, recent developments point to a clear and pressing need to explore alternative AD treatment strategies since clinical trials aimed at attenuating Aβ generation or promoting the peptides clearance have limited efficacies and/or serious side effects [106-113]. Thus, development of AD lesion-specific metal complexing agents combined with nanotechnology have made promising breakthroughs. In addition to attenuate Aβ and tau associated pathology [67,77], clinical trials have shown their efficacy in attenuating other AD symptoms [61,65] and low toxicity [67]. Overall, aforementioned compounds show considerable promise as drug delivery vehicles and diagnostic tools for AD.

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Conflict of Interest

The authors declare no conflict of interest.

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