Biometals and Their Therapeutic Implications in Alzheimer's Disease

ARTICLE *in* JOURNAL OF THE AMERICAN SOCIETY FOR EXPERIMENTAL NEUROTHERAPEUTICS · OCTOBER 2014

Impact Factor: 5.05 · DOI: 10.1007/s13311-014-0312-z · Source: PubMed

Neurotherapeutics
DOI 10.1007/s13311-014-0312-z

REVIEW

Biometals and Their Therapeutic Implications in Alzheimer's Disease

Scott Ayton · Peng Lei · Ashley I. Bush

Abstract No disease modifying therapy exists for Alzheimer's disease (AD). The growing burden of this disease to our society necessitates continued investment in drug development. Over the last decade, multiple phase 3 clinical trials testing drugs that were designed to target established disease mechanisms of AD have all failed to benefit patients. There is, therefore, a need for new treatment strategies. Changes to the transition metals, zinc, copper, and iron, in AD impact on the molecular mechanisms of disease, and targeting these metals might be an alternative approach to treat the disease. Here we review how metals feature in molecular mechanisms of AD, and we describe preclinical and clinical data that demonstrate the potential for metal-based drug therapy.

Keywords Alzheimer's disease · Iron · Copper · Zinc · Chelation therapy

Introduction

Alzheimer's disease (AD) is characterized by profound dementia, commonly presenting as impairment to memory, language, perceptual skills, attention, constructive abilities, orientation, problem solving, and functional abilities. At an anatomic level, AD manifests with neuronal and synaptic loss, particularly in the hippocampus, frontal cortex, temporal lobe, parietal lope, and cingulate gyros. The aging population demographic of our society will inevitably lead to increased AD incidence in the future, adding to an already substantial financial and social burden. Despite an extensive effort by the scientific community, there remains no disease modifying therapy to halt or slow the decline of patients with AD.

The current dominant therapeutic approaches for AD have emerged from the amyloid cascade hypothesis. This hypothesis was raised in 1992 [1], and encapsulates the dominant thinking in AD research. This hypothesis emphasizes the importance of β-amyloid (Aβ), the major component of senile plaques [2], as an upstream factor in AD pathogenesis, and posits that Aβ initiates a cascade of toxicity, which recruits tau, the major component of tangles in AD pathology [3], and consequently causes neuronal dysfunction, neurotransmitter deficits, and, ultimately, neuron death. Aβ is generated by cleavage of amyloid precursor protein (APP) [4], which can occur via 2 sequential pathways: the nonamyloidogenic and amyloidogenic pathways. The first cleavage event, by α-secretase or β-secretase, determines whether Aβ is generated; the proceeding cleavage by γ-secretase determines the species of Aβ to be generated. β-secretase activity is contributed mostly by an integral membrane aspartic protease named β-site APP-cleaving enzyme 1 [5], and γ-secretase is a protease complex consisting nicastrin, presenilin enhancer 2, anterior pharynx-defective 1 and presenilin, which harbors the active site [6].

The implication of the amyloid cascade hypothesis is that targeting Aβ should prevent molecular pathology of AD and alter the course of the disease. Accordingly, Aβ-based therapeutics have been extensively investigated in preclinical models and clinical trials. Most prominently, phase 3 trials have been performed on drugs that lower Aβ by inhibiting its production [7, 8] and lowering its levels by immunotherapy [9, 10], all of which failed to reach their respective clinical endpoints. There is an urgent need, therefore, for fresh approaches to treat AD.

Scott Ayton and Peng Lei contributed equally to this work.

S. Ayton · P. Lei · A. I. Bush (✉)
Oxidation Biology Unit, Florey Institute of Neuroscience and Mental Health, The University of Melbourne, 30 Royal Parade, Parkville 3052, VIC, Australia
e-mail: ashleyib@unimelb.edu.au

Published online: 30 October 2014

We have for several years been investigating the role of transition metals (e.g., iron, copper, and zinc) in the pathogenesis of AD, and whether targeting metals could impact on the course of disease. There is dysregulation of the homeostatic mechanisms of iron, copper, and zinc in AD, and these metals can bind pathologically to Aβ and tau proteins [11]. We, and others, have integrated the metal hypothesis of AD with the amyloid cascade hypothesis, and preclinical and clinical data demonstrate that metals might be a more tractable drug target to address underlying disease mechanisms of AD. In this review, we will describe the setbacks with Aβ-targeted drug discovery and discuss current and future opportunities for metal-based drug therapy.

Clinical Trial Setbacks for Drugs That Target Aβ

Despite extensive empirical data demonstrating the upstream role for Aβ in AD toxicity, targeting this peptide by multiple avenues has not been effective in clinical trials [12]. AN-1792 was the first active immunotherapy strategy for AD. In PDAPP mice (overexpressing a disease-related mutant APP), immunization prevented and rescued the AD behavioral and pathological phenotypes [13]. The multinational phase 2a trial in people with mild-to-moderate AD was suspended when 6 % of patients had a side effect, including 4 with aseptic meningoencephalitis [14]. In a follow-up analysis of the phase 1 clinical trial, the treatment group had less plaque burden at autopsy (some even had complete plaque removal) but dementia presentation was not different between the treatment and placebo group [15]. In the phase 2 trial, those who responded to the immunization (anti-AN1792 titers >1 : 2200) had improved cognition and attenuated brain volume loss compared with placebo patients [16].

A few passive immunotherapy approaches have undergone phase 3 clinical assessment, including bapineuzumab, gantenerumab, and solanezumab. Bapineuzumab appeared, in a 12-month phase 1 study, to be safe in patients with mild-to-moderate AD. Two phase 2 studies were conducted, showing no significant benefits in cognitive measurements [17], but reduced cortical amyloid burden [18]. Some cognitive and functional improvements were observed in a subset of apolipoprotein E ε4 noncarriers [17], which prompted 4 additional phase 3 trials. Two completed trials failed to show effect on either cognitive or functional outcomes, and the other trials were discontinued [10]. Gantenerumab was shown to bind to cerebral Aβ in APP/presenilin 1 (PS1) transgenic mouse model, to reduce amyloid burden in mouse brain, and to prevent the formation of new plaque [19]. Roche started a phase 2 trial (clinical trial number: NCT01224106) and the Dominantly Inherited Alzheimer Network initiated a phase 2/3 trial (clinical trial number: NCT01760005) to test for efficacy. These trials are ongoing. Solanezumab was developed by Lily, and in phase 2 clinical trials there was a significant dose-dependent increase in Aβ42 in cerebrospinal fluid but no indication of clinical benefit [20]. In phase 3, 2 trials were performed and no benefits in cognitive functions of patients with AD were found [9]. However, a secondary analysis of patients with mild AD pooled from both trials showed a significant effect on cognition, promoting the Dominantly Inherited Alzheimer Network's 5-year phase 2/3 trial (clinical trial number: NCT01760005), which is currently ongoing.

Small molecules that could modulate γ-secretase, and thus production of Aβ, have been tested in phase 3 clinical trials. A γ-secretase inhibitor, semagacestat, was tested in a phase 2 trial and a significant reduction in plasma Aβ40 was found [21]. However, the phase 3 trial was halted after interim analysis showed worsening of cognition and increased incidence of skin cancer [8], which was predicted previously [22]. Later, a γ-secretase modulator, tarenflurbil, was tested in a 12-month phase 2 study in people with mild-to-moderate AD. This trial found no effect on cognition, although with higher dose and patients with mild AD there were small benefits [23]. This encouraged a phase 3 clinical trial in 1600 patients with AD but found no beneficial effects in cognitive function and quality of life [7], which resulted in termination of 2 additional phase 3 trials and further development of this compound. Currently, there are still a few phase 3 clinical trials ongoing for AD targeting Aβ, including the abovementioned gantenerumab and solanezumab, and a β-secretase inhibitor, MK-8931.

Challenges in Targeting Amyloid

Despite the setbacks in drug discovery, the amyloid cascade hypothesis still represents the best explanation of the pathological phenomenon in AD. However, direct targeting of Aβ is difficult owing to the unidentified toxic species of Aβ and its complex interaction with tau.

Various Aβ species exist within the brain, including Aβ1-39, Aβ1-40, Aβ1-42, Aβ1-43, and Aβ3-42. The toxic species of Aβ is still unknown and therefore we do not have a clear target for pharmacotherapy; this is one possible explanation of why Aβ-based drugs have not been effective so far. Traditionally, it was believed that Aβ1-42 is predominately responsible for toxicity observed in AD. Two key observations support this theory: 1) familial AD mutations in APP elevate the expression of Aβ1-42 [24, 25]; and 2) Aβ1-42 is more prone to aggregation and is more toxic than Aβ1-40 [26, 27]. However, it was reported that pyroglutamate-Aβ, which is also presented in AD plaques, could also be the toxic isoform of Aβ [28–31]. Pyroglutamate-Aβ is more prone to aggregation compared with Aβ1-42 [29]. Mice overexpressing this peptide showed a selective hippocampal

neurodegeneration [31], and passive immunization against this peptide reduces the amyloid burden in an APP/PS1 double-transgenic mouse model [30]. In addition, Aβ1-43, generated by a disease-related PS1 mutant, was recently profiled as an overlooked neurotoxic isoform as this species was shown to have a higher propensity to aggregate and was more toxic than Aβ1-42 [32].

The various isoforms alter the aggregation propensity and therefore its solubility. In AD, Aβ exists in both soluble and insoluble forms, with the later associated with plaques. Plaques (containing insoluble amyloid fibrils) were initially thought to be the toxic Aβ source in the earlier version of amyloid cascade hypothesis [1]; however, subsequent findings suggest that soluble Aβ oligomers are more likely to be the source of Aβ toxicity, while insoluble fibres are inert (for a review see [33]). Numerous studies have postulated specific toxic forms of Aβ; however, the field is without a clear conclusion. A higher molecular weight Aβ oligomer (about 56 kDa) was purified from Tg2576 mice (an APP mutant mouse model of AD) and demonstrated to be toxic [34]. It was then found that naturally secreted oligomers (a mixture of trimers and dimers) can inhibit LTP in vivo [35] and disturb cognitive function in mice [35, 36]. The toxic species of these "naturally secreted oligomers" was narrowed down to the "Aβ dimer" (based on its 8-kDa molecular weight), as that "Aβ dimer" isolated from AD brains is capable of impairing memory in mice [37]. However this so-called dimer was identified to be an APP fragment containing Aβ [38].

Therefore, the toxic species of Aβ in AD is still under debate. It is unknown if any of the drugs tested in clinical trial, which target alternative cleavage of APP, or target Aβ species immunologically, have been effective inor isolating the toxic Aβ isoform. Moreover, it was recently shown that not all the therapeutic antibodies bind to Aβ species in plasma or post-mortem brain samples, which potentially explains why they were not effective in clinical trial [39].

It is also still unknown how Aβ induces toxicity to neurons, which has slowed the development of drugs that target intracellular consequences of Aβ toxicity. It is generally accepted that tau protein is an intracellular mediator of Aβ toxicity. Aβ induces tau hyperphosphorylation, aggregation, and enhances tau toxicity. Treating cell cultures with multiple forms of Aβ, including soluble Aβ1-42 [40], Aβ oligomer [41], and Aβ fibril [42] induces tau hyperphosphorylation. Aβ-induced tau hyperphosphorylation consequently caused microtubule disassembly [41, 43], reduction of total soluble tau and an increase in tau fragments [44], missorting of tau into dendritic areas [41], activation of the nuclear transcription factor of activated T cells' apoptotic pathways [45, 46], and cell toxicity [41, 44]. 2xTg (tau and APP mutant) or 3xTg (tau, APP and PS mutant) mice exhibit enhanced neurofibrillary tangle formation [47–50], which was attributed to activation of glycogen synthase kinase (GSK)-3β and Cdk5 [49, 51], and inhibited proteasome activity [50]. Additional overexpression of tau does not enhance Aβ pathology in vivo [51], supporting the notion that tau pathology is downstream of Aβ. The effects on tau seen in the 2xTg mouse can be recapitulated by direct Aβ fibril injection, implying that Aβ is the minimal fragment of APP required to transform tau [52]. Similarly, Aβ vaccination reduces both Aβ and tau pathology and protects against neuron loss [53–55].

Interestingly, Aβ-induced cytotoxicity was shown to be tau-dependent in primary neuronal culture with either genetic depletion of tau, or using an undifferentiated neuronal culture (which has a lower tau level than differentiated neurons) [56, 57]. Tau knockout studies revealed that the Aβ-induced axonal transport deficits and impairment of hippocampal LTP were mediated by tau [58, 59]. Reduction of endogenous tau was also shown to ameliorate cognitive behavioral deficits and the death rate of APP overexpressing mice [60–62]. However, while young $APP^{+/+}tau^{-/-}$ mice (4–7 months old) exhibit better cognitive function [60, 61], aged $APP^{+/+}tau^{-/-}$ mice (12 months old or older) showed enhanced degeneration [63], indicating that loss of tau function alone may be toxic. Further investigations found that loss of tau induces parkinsonism and dementia phenotypes [64–66], suggesting that excessive lowering of tau should be avoided in therapeutic strategies for AD.

While substantial laboratory data show a role of tau in mediating Aβ-induced cytotoxicity, tau-based therapies have yet to be explored in phase 3 clinical trials. Targeting tau, or other downstream mediators of Aβ toxicity, might be a more effective approach to treating the disease. It has been shown that AD plaque burden begins 20 years before the onset of disease [67], so targeting Aβ when the disease is clinically manifest might be too late in the temporal sequence of the disease.

While the amyloid cascade hypothesis of AD still maintains broad support, the failure of drugs that target Aβ has led to growing skepticism of this explanation of AD. Indeed, positron emission tomographic (PET) imaging data have shown that amyloid can be removed from the brain by immunotherapy, and this does not translate into improved clinical outcomes [68]. This supports the hypothesis that the presence of amyloid plaque in the brain reflects the presence of the disease but is not directly involved in neurodegeneration. It has been suggested that amyloid plaque is a protective response to underlying disease processes, as the presence of plaque inversely correlates with oxidative burden and synaptic loss [69, 70]. Copper enhances Aβ cytotoxicity to cultured cells, which is ameliorated by co-incubation with zinc [69], which rapidly precipitates the protein [71]. Plaque may therefore be a protective response in AD by performing metal sequestration [72]. Targeting plaque "tombstones", therefore, would not affect disease progression, but correcting the underlying disturbance in metal homeostasis could be a more tractable therapeutic avenue.

Metals in AD

While the amyloid cascade hypothesis may yet sill prove to be valid, as described above, there are many challenges to targeting Aβ, and no approach so far has been effective in patients. Here, we present a hypothesis that targeting metals in AD might be an alternative, more tractable, therapeutic strategy. We outline the studies describing the manifest changes to metals (zinc, copper, iron) in the AD brain, and how changes to metals might impact on the established disease mechanisms of AD. Finally, we describe the outcomes of various preclinical and clinical attempts of targeting metals in AD.

Zinc

Bulk tissue analysis of the postmortem AD brain has generated inconsistent changes between AD and controls [73–77], which might reflect different anatomic areas examined and different sample preparations (e.g., tissue fixation affects zinc measurement [78]). But analysis of bulk tissue zinc content is likely to miss the most interesting changes to zinc in the AD brain. In AD, there is a redistribution of zinc into extracellular plaques and surrounding neuropil [71, 79–82]. Zinc binds Aβ residues 6–28 causing rapid aggregation and precipitation of the peptide [71, 83]; thus, co-deposition of zinc with Aβ could initiate plaque formation in the disease.

Zinc-induced plaque formation in disease is also supported by the anatomic distribution of plaque and zinc in the brain. Plaque formation only occurs in neocortical regions of AD-affected brains, but Aβ is expressed in all brain regions. The distribution of plaque closely aligns with the expression of a key zinc-transporting protein of glutametergic neurons, ZnT3. ZnT3 traffics zinc into synaptic vesicles and zinc is released into the synapse upon exocytosis. APP transgenic mice crossed with mice lacking ZnT3 were shown to have reduced plaque burden compared with single transgenic mice [84], which demonstrates the contribution of endogenous zinc to amyloid burden in AD. Zinc "trapping" in amyloid plaque is also likely to be deleterious to synaptic function as loss of synaptic zinc in ZnT3 KO mice causes impaired cognition, recapitulating AD [85]. Synaptic zinc deficiency is further exacerbated in AD by lower expression of ZnT3 [82].

Zinc also interferes with Aβ processing. A disintegrin and metalloproteinase domain-containing protein 10, the α-secretase involved in the physiological processing of APP, is a zinc-dependent enzyme and zinc increases APP proteolysis [86], while zinc inhibits γ-secretase activity [87]. Zinc also increase PS1 expression, and presenilin protein also facilitates cellular zinc uptake [88]. Finally, Aβ bound to zinc is resistant to proteolysis by zinc-dependent matrix metalloproteases [89, 90].

Zinc might also impact on tau-related neurotoxicity in AD. Zinc can bind to tau and cause its aggregation [91–93]. Zinc is elevated in neurons with neurofibrillary tangle pathology [79], which could alter tau translation and phosphorylation by GSK-3β, protein kinase B, extracellular regulated kinase 1/2 and c-Jun N-terminal kinase [94–96].

Iron

Iron elevation in affected areas of brain is a feature of a number of neurodegenerative disorders, including Parkinson's disease and AD [97, 98]. Iron elevation in AD brains, first demonstrated in 1953 [99], is a consistently reported finding [99–108]. Neuronal iron deposition causes oxidative stress via the Fenton reaction, which might contribute to elevated oxidative stress observed in the AD brain [109]. Iron-induced oxidative stress has been shown to initiate several apoptotic signaling pathways in neurons [110], and damage proteins such as Ca^{2+}-ATPase [111–114], glutamate transporter [115–117], apolipoprotein E [118, 119], and Na^{+}/K^{+}-ATPase [111, 114, 120, 121], as well as *N*-methyl-D-aspartate (NMDA) receptor [122–124], and lipids such as cholesterol [125–127], ceramides [128, 129], and unsaturated fatty acids [130–133], as well as sphingomyelin [134, 135]. Oxidative damage to proteins and lipids by iron can cause synaptic dysfunction and neuronal cell death [136].

Elevated iron can also cause cell death independently of free radical toxicity by causing ferroptosis, a type of Ras-related (apoptosis independent) cell death pathway [137]. This cell death pathway is only beginning to be explored in neurodegenerative diseases, but could have therapeutic implications as ferroptosis can prevented by iron chelation or iron uptake inhibition [138, 139].

The overload of iron in AD may also lead to increased Aβ production by increasing the expression of APP, and altering its processing. APP has a 5′ untranslated region iron-responsive element [140]; in conditions of high iron (e.g., AD) restricted translation of APP by iron-responsive proteins is disinhibited, leading to increased translation of the transcript. High iron conditions also leads to increased processing of APP [141], which leads to accelerated degeneration in a mouse model of AD [142]. Iron is particularly enriched in plaques [99], and iron causes the aggregation of Aβ in vitro [143, 144]. Iron-stimulated aggregates of Aβ are cytotoxic in vitro [145–149].

Tau also binds to iron [150, 151], which causes it to aggregate [152], possibly depositing in vivo as iron-rich tangles in AD brains [108]. Iron treatment to cultured neurons increases tau phosphorylation [153–156], so upstream iron elevation might increase pathological tau in AD. Total tau levels are also decreased in AD cortex [157–160], and we showed that loss of tau expression causes iron- and age-dependent cognitive loss and cortical atrophy in mice [64]. Tau is required for APP trafficking to the neuronal membrane [64]. APP binds to ferroportin in the neuronal membrane and

facilitates iron export of neurons [106, 161], which is neuroprotective [162], so reduced tau or APP levels could lead to iron retention in neurons that is observed in AD.

Copper

Copper is also a redox-active metal that can catalyse the formation of the hydroxyl radical via the Fenton reaction [163], and can cause free radical-mediated damage and cell death in the same way as iron [163]. In the presence of amyloid copper undergoes redox cycling, which enhances the potential for copper induced toxicity in AD [164–166]. This is important because copper is increased in amyloid; however, copper levels are decreased in AD neuronal tissue [77], which could deprive copper-binding proteins such as superoxide dismutase and ceruloplasmin of the metal, which can impair their function. Superoxide dismutase is a major copper binding protein and antioxidant in neurons, which utilizes copper to convert the superoxide free radical into hydrogen peroxide [167]. Ceruloplasmin is another major copper-binding protein, which functions as a ferroxidase to promote iron export [168, 169]. The protein requires copper binding to perform this function, and low copper levels could lead to iron accumulation by impairing ceruloplasmin-mediated iron export [170].

Copper can also accelerate Aβ aggregation but, unlike zinc, copper induces oligomer, rather than fibril, structures [171–175]. Toxicity of Aβ oligomers can be attenuated in cultures by copper chelation [176, 177]. The mechanism of copper-Aβ induced cytotoxicity might involve oxidative stress, as the complex catalytically generates hydrogen peroxide [178–180]. The binding of copper to Aβ is likely to be a factor in AD because copper, too, is enriched in amyloid plaque [82]. Copper and Aβ are both released into the glutamatergic synaptic cleft, where hypoactiivty of the glutamate receptor, NMDA [181], is thought to contribute to cognitive decline in AD [182]. The co-release of copper and Aβ might make them available for pathological interactions. The concentration of copper in the glutamatergic synaptic cleft approximates 15 μM [183, 184], and copper in the synapse can modulate glutamateric signaling by causing s-nitrosylation-dependent inhibition of the NMDA signaling [185, 186], contributing to glutamatergic dysfunction in the disease [181].

Tau also binds copper in vitro causing it to aggregate and generate hydrogen peroxide [187–191]. This recapitulates what is observed in AD brains where copper-containing neurofibrillary tangles area a source of oxidative stress [192]. In transgenic mice overexpressing mutant APP, presenilin and tau, copper exposure accelerates tau hyperphosphorylation [193]. However, in mice that overexpress only APP and presenilin, copper delivery drugs have been shown to reduce GSK-3β-dependent tau phosphorylation [194].

Targeting Metals to Treat AD

Clioquinol and PBT2

5-Chloro-7-iodo-quinolin-8-ol (Clioquinol) is an antiparasitic agent that was withdrawn from clinical use owing to a speculated severe side effect, subacute myelo-optico-neuropathy (SMON). This side effect was only observed in Japan [195], and the association between SMON and clioquinol has since been questioned [196]. Indeed, no subsequent clinical trials have observed SMON in participants treated with clioquinol. Clioquinol has a moderate affinity for iron, copper, and zinc (Kd_{Cu} is 1.2×10^{-10} M, Kd_{Zn} is 7×10^{-8} M) [197], and for this reason it was explored as a drug for AD. Although it was initially considered a chelator [198–201], it has more recently been characterized as a copper/zinc ionophore, which functions to redistribute these metals into cells [196, 202–206]. Clioquinol is still considered a moderate iron chelator as it has been shown to lower iron levels in animal models of iron overload [64, 148, 207–210], and has not been shown to redistribute iron into cells using ionophore assays.

We hypothesized that clioquinol might correct metal miscompartmentalization in AD by redistributing zinc and copper from extracellular plaque (preventing Aβ aggregation) into zinc- and copper-deficient neurons (to restore normal function). Clioqinol might also remove iron from overload neurons. Indeed, clioquinol has shown efficacy in patients with AD and models in of disease. Clioquinol inhibits Aβ oligomer formation and protects against cell loss in an Aβ-injection model [211–213]. Nine weeks of oral clioquinol treatment in an AD mice lowered plaque burden and improved cognitive performance [196, 214]. In a phase 2 clinical trial of 32 patients, clioquinol prevented cognitive deterioration (Alzheimer's Disease Assessment Scale-cognitive) and lowered plasma Aβ42 levels over 36 weeks [215]. While these data were promising, complications with the large-scale manufacturing of the compound made further development of this drug unviable.

PBT2 is a new 8-hydroxyquinoline based on the clioquinol chemical scaffold and has shown, by restoring cognitive function after 11 days of treatment, even greater efficacy in a mouse model of AD [203]. In a phase 2a clinical trial of 78 patients, 12 weeks of PBT2 treatment caused a dose-dependent lowering of cerebrospinal fluid Aβ and improved 2 measures of executive function at the highest dose in the study (250 mg) [216, 217]. In a recent phase 2b clinical trial of 1 year's duration, PBT2 did not show favorable clinical outcomes. While this was a longer study than then original phase 2a trial, the number of trial participants was less; indeed, there were only 15 patients allocated to the placebo group in the study. The primary endpoint to the phase 2b trial was improved Pittsburgh compound B–PET scan compared with placebo patients. While patients on PBT2 had a lowering

Pittsburgh compound B–PET signal, the result was also confounded by an inexplicable reduction in the placebo group. The trial also did not report improvement to any cognitive readout included in the study. The trial has been extended for a further 12 months to analyze cognitive and structural magnetic resonance measures, and is expected to conclude in late 2014.

PBT2 has also shown promise for Huntington's disease (HD). HD is a rare genetic disorder characterized by chorea motor dysfunction and dementia. In the R6/2 mouse model of HD, PBT2 increased lifespan and improved motor performance [218]. In a phase 2 clinical trial of 109 patients with HD across 20 sites in the USA and Australia, PBT2 treatment over 26 weeks improved measures of executive function, but without improving motor disability. PBT2 is scheduled to undergo a phase 3 study for HD.

Bis(thiosemicarbazone) Ligands

Agents that directly deliver copper have also been explored as therapeutics for AD given that both clioquinol and PBT2 function, in part, to deliver copper to neurons. The copper-containing bis(thiosemicarbazone) compound Cu^{II}GTSM, has been shown to lower Aβ levels, GSK3β activity, and phosphorylated tau levels in cell culture and APP/PS1 transgenic mice [194, 219], which accompanied improved cognition in the Y-maze assay. A related compound, Cu^{II}ATSM, was not beneficial to the APP/PS1 transgenic model of AD, but conferred neuroprotection in 4 animal models of PD [220], which is also complicated by copper deficiency [168].

Iron Chelators

The first AD clinical trial investigating a metal-based drug was performed in 1991. Deferoxamine is a bacterial siderophore that has a strong affinity for iron, and also aluminium. Indeed, when the trial was performed it was hypothesized that deferoxamine would target aluminium toxicity in AD. In a single-blind trial of 48 AD patients, deferoxamine reduced the rate of cognitive decline over a 24-month period [221]. This encouraging trial data has not led to further AD clinical development of compounds that target iron. However, a recent Parkinson's disease clinical trial of the iron chelator, deferiprone, showed improvement in motor function over an 18-month period [222].

Conclusions

Despite extensive investment, the AD research community has been collectively frustrated by the lack of clinical outcomes and research breakthroughs that have been translatable into humans. The challenge before us, of developing new treatments for AD, remains as extensive as it is urgent. Metal-based therapies for AD might be a therapeutic alternative that could confer benefits to patients. Extensive preclinical and clinical data underlie the rational of metal based therapeutic approaches, and large-scale phase 3 trials are warranted.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

References

1. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science 1992;256:184-185.
2. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci U S A 1985;82:4245-4249.
3. Kosik KS, Joachim CL, Selkoe DJ. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc Natl Acad Sci U S A 1986;83:4044-4048.
4. Tanzi RE, Gusella JF, Watkins PC, et al. Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. Science 1987;235:880-884.
5. Yan R, Bienkowski MJ, Shuck ME, et al. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. Nature 1999;402:533-537.
6. Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, Selkoe DJ. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature 1999;398:513-517.
7. Green RC, Schneider LS, Amato DA, et al. Effect of tarenflurbil on cognitive decline and activities of daily living in patients with mild Alzheimer disease: a randomized controlled trial. JAMA 2009;302:2557-2264.
8. Doody RS, Raman R, Farlow M, et al. A phase 3 trial of semagacestat for treatment of Alzheimer's disease. N Engl J Med 2013;369:341-350.
9. Doody RS, Thomas RG, Farlow M, et al. Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. N Engl J Med 2014;370:311-321.
10. Salloway S, Sperling R, Fox NC, et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. N Engl J Med 2014;370:322-333.
11. Ayton S, Lei P, Bush AI. Metallostasis in Alzheimer's disease. Free Radic Biol Med 2013;62:76-89.
12. Karran E, Hardy J. A critique of the drug discovery and phase 3 clinical programs targeting the amyloid hypothesis for Alzheimer disease. Ann Neurol 2014;76:185-205.
13. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999;400:173-177.
14. Hock C, Konietzko U, Streffer JR, et al. Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. Neuron 2003;38:547-554.
15. Holmes C, Boche D, Wilkinson D, et al. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. Lancet. 2008;372:216-223.

16. Vellas B, Black R, Thal LJ, et al. Long-term follow-up of patients immunized with AN1792: reduced functional decline in antibody responders. Curr Alzheimer Res 2009;6:144-151.
17. Salloway S, Sperling R, Gilman S, et al. A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. Neurology 2009;73:2061-2070.
18. Rinne JO, Brooks DJ, Rossor MN, et al. 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. Lancet Neurol 2010;9:363-372.
19. Bohrmann B, Baumann K, Benz J, et al. Gantenerumab: a novel human anti-Abeta antibody demonstrates sustained cerebral amyloid-beta binding and elicits cell-mediated removal of human amyloid-beta. J Alzheimers Dis 2012;28:49-69.
20. Farlow M, Arnold SE, van Dyck CH, et al. Safety and biomarker effects of solanezumab in patients with Alzheimer's disease. Alzheimers Dement 2012;8:261-271.
21. Fleisher AS, Raman R, Siemers ER, et al. Phase 2 safety trial targeting amyloid beta production with a gamma-secretase inhibitor in Alzheimer disease. Arch Neurol 2008;65:1031-1038.
22. Zhang Y-W, Wang R, Liu Q, Zhang H, Liao F-F, Xu H. Presenilin/gamma-secretase-dependent processing of beta-amyloid precursor protein regulates EGF receptor expression. Proc Natl Acad Sci USA 2007;104:10613-10618.
23. Wilcock GK, Black SE, Hendrix SB, et al. Efficacy and safety of tarenflurbil in mild to moderate Alzheimer's disease: a randomised phase II trial. Lancet Neurol 2008;7:483-493.
24. Goate A, Chartier-Harlin MC, Mullan M, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 1991;349:704-706.
25. Borchelt DR, Thinakaran G, Eckman CB, et al. Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. Neuron 1996;17:1005-1013.
26. Jan A, Gokce O, Luthi-Carter R, Lashuel HA. The ratio of monomeric to aggregated forms of Abeta40 and Abeta42 is an important determinant of amyloid-beta aggregation, fibrillogenesis, and toxicity. J Biol Chem 2008;283:28176-28189.
27. Kuperstein I, Broersen K, Benilova I, et al. Neurotoxicity of Alzheimer's disease Aβ peptides is induced by small changes in the Aβ42 to Aβ40 ratio. EMBO J 2010;29:3408-3420.
28. Schilling S, Zeitschel U, Hoffmann T, et al. Glutaminyl cyclase inhibition attenuates pyroglutamate Abeta and Alzheimer's disease-like pathology. Nat Med 2008;14:1106-1111.
29. Schlenzig D, Ronicke R, Cynis H, et al. N-Terminal pyroglutamate formation of Abeta38 and Abeta40 enforces oligomer formation and potency to disrupt hippocampal long-term potentiation. J Neurochem 2012;121:774-784.
30. Frost JL, Liu B, Kleinschmidt M, Schilling S, Demuth HU, Lemere CA. Passive immunization against pyroglutamate-3 amyloid-beta reduces plaque burden in Alzheimer-like transgenic mice: a pilot study. Neurodegener Dis 2012;10:265-270.
31. Alexandru A, Jagla W, Graubner S, et al. Selective hippocampal neurodegeneration in transgenic mice expressing small amounts of truncated Abeta is induced by pyroglutamate-Abeta formation. J Neurosci 2011;31:12790-12801.
32. Saito T, Suemoto T, Brouwers N, et al. Potent amyloidogenicity and pathogenicity of Aβ43. Nat Neurosci 2011;14:1023-1032.
33. Karran E, Mercken M, De Strooper B. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat Rev Drug Discov 2011;10:698-712.
34. Lesné S, Koh MT, Kotilinek LA, et al. A specific amyloid-beta protein assembly in the brain impairs memory. Nature 2006;440:352-357.
35. Walsh DM, Klyubin I, Fadeeva JV, et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 2002;416:535-539.
36. Cleary JP, Walsh DM, Hofmeister JJ, et al. Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. Nat Neurosci 2005;8:79-84.
37. Shankar GM, Li S, Mehta TH, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 2008;14:837-842.
38. Welzel AT, Maggio JE, Shankar GM, et al. Secreted amyloid beta-proteins in a cell culture model include N-terminally extended peptides that impair synaptic plasticity. Biochemistry 2014;53:3908-3921.
39. Watt AD, Crespi GA, Down RA, et al. Anti-Abeta antibody target engagement: a response to Siemers et al. Acta Neuropathol 2014;128:611-614.
40. Thornton C, Bright NJ, Sastre M, Muckett PJ, Carling D. AMP-activated protein kinase (AMPK) is a tau kinase, activated in response to amyloid beta-peptide exposure. Biochem J 2011;434:503-512.
41. Zempel H, Thies E, Mandelkow E, Mandelkow E-M. Abeta oligomers cause localized Ca(2+) elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. J Neurosci 2010;30:11938-11950.
42. Busciglio J, Lorenzo A, Yeh J, Yankner BA. beta-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. Neuron 1995;14:879-888.
43. King ME, Kan H-M, Baas PW, Erisir A, Glabe CG, Bloom GS. Tau-dependent microtubule disassembly initiated by prefibrillar beta-amyloid. J Cell Biol 2006;175:541-546.
44. Reifert J, Hartung-Cranston D, Feinstein SC. Amyloid {beta}-mediated cell death of cultured hippocampal neurons reveals extensive tau fragmentation without increased full-length tau phosphorylation. J Biol Chem 2011;286:20797-20811.
45. Ayton S, Lei P. The Aβ-induced NFAT apoptotic pathway is also activated by GSK-3 inhibition: Implications for Alzheimer therapeutics. J Neurosci 2012;32:9454-9456.
46. Hudry E, Wu HY, Arbel-Ornath M, et al. Inhibition of the NFAT pathway alleviates amyloid Beta neurotoxicity in a mouse model of Alzheimer's disease. J Neurosci 2012;32:3176-3192.
47. Hurtado DE, Molina-Porcel L, Iba M, et al. A{beta} accelerates the spatiotemporal progression of tau pathology and augments tau amyloidosis in an Alzheimer mouse model. Am J Pathol 2010;177:1977-1988.
48. Lewis J, Dickson DW, Lin WL, et al. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science 2001;293:1487-1491.
49. Terwel D, Muyllaert D, Dewachter I, et al. Amyloid activates GSK-3beta to aggravate neuronal tauopathy in bigenic mice. Am J Pathol 2008;172:786-798.
50. Tseng BP, Green KN, Chan JL, Blurton-Jones M, LaFerla FM. Abeta inhibits the proteasome and enhances amyloid and tau accumulation. Neurobiol Aging 2008;29:1607-1618.
51. Oddo S, Caccamo A, Cheng D, Jouleh B, Torp R, LaFerla FM. Genetically augmenting tau levels does not modulate the onset or progression of Abeta pathology in transgenic mice. J Neurochem 2007;102:1053-1063.
52. Götz J, Chen F, van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science 2001;293:1491-1495.
53. Ma QL, Lim GP, Harris-White ME, et al. Antibodies against beta-amyloid reduce Abeta oligomers, glycogen synthase kinase-3beta activation and tau phosphorylation in vivo and in vitro. J Neurosci Res 2006;83:374-384.
54. Wilcock DM, Gharkholonarehe N, Van Nostrand WE, Davis J, Vitek MP, Colton CA. Amyloid reduction by amyloid-beta vaccination also reduces mouse tau pathology and protects from neuron loss in two mouse models of Alzheimer's disease. J Neurosci 2009;29:7957-7965.

55. Oddo S, Billings L, Kesslak JP, Cribbs DH, LaFerla FM. Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. Neuron 2004;43:321-332.
56. Liu T, Perry G, Chan HW, et al. Amyloid-beta-induced toxicity of primary neurons is dependent upon differentiation-associated increases in tau and cyclin-dependent kinase 5 expression. J Neurochem 2004;88:554-563.
57. Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A. Tau is essential to beta -amyloid-induced neurotoxicity. Proc Natl Acad Sci U S A 2002;99:6364-6369.
58. Vossel KA, Zhang K, Brodbeck J, et al. Tau reduction prevents A{beta}-induced defects in axonal transport. Science 2010;330:198.
59. Shipton OA, Leitz JR, Dworzak J, et al. Tau protein is required for amyloid {beta}-induced impairment of hippocampal long-term potentiation. J Neurosci 2011;31:1688-1692.
60. Roberson ED, Scearce-Levie K, Palop JJ, et al. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. Science 2007;316:750-754.
61. Ittner LM, Ke YD, Delerue F, et al. Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. Cell 2010;142:387-397.
62. Roberson ED, Halabisky B, Yoo JW, et al. Amyloid-β/Fyn-induced synaptic, network, and cognitive impairments depend on tau levels in multiple mouse models of Alzheimer's disease. J Neurosci 2011;31:700-711.
63. Dawson HN, Cantillana V, Jansen M, et al. Loss of tau elicits axonal degeneration in a mouse model of Alzheimer's disease. Neuroscience 2010;169:516-531.
64. Lei P, Ayton S, Finkelstein DI, et al. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. Nat Med 2012;18:291-295.
65. Ma QL, Zuo X, Yang F, et al. Loss of MAP function leads to hippocampal synapse loss and deficits in the Morris Water Maze with aging. J Neurosci 2014;34:7124-7136.
66. Lei P, Ayton S, Moon S, et al. Motor and cognitive deficits in aged tau knockout mice in two background strains. Mol Neurodegener 2014;9:29.
67. Villemagne VL, Burnham S, Bourgeat P, et al. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. Lancet Neurol 2013;12:357-367.
68. Lemere CA, Masliah E. Can Alzheimer disease be prevented by amyloid-beta immunotherapy? Nat Rev Neurol 2010;6:108-119.
69. Cuajungco MP, Goldstein LE, Nunomura A, et al. Evidence that the beta-amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of abeta by zinc. J Biol Chem 2000;275:19439-19442.
70. Lue LF, Kuo YM, Roher AE, et al. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am J Pathol 1999;155:853-862.
71. Bush AI, Pettingell WH, Multhaup G, et al. Rapid induction of Alzheimer A beta amyloid formation by zinc. Science 1994;265:1464-1467.
72. Atwood CS, Robinson SR, Smith MA. Amyloid-beta: redox-metal chelator and antioxidant. J Alzheimers Dis 2002;4:203-214.
73. Danscher G, Jensen KB, Frederickson CJ, et al. Increased amount of zinc in the hippocampus and amygdala of Alzheimer's diseased brains: a proton-induced X-ray emission spectroscopic analysis of cryostat sections from autopsy material. J Neurosci Methods 1997;76:53-59.
74. Panayi AE, Spyrou NM, Iversen BS, White MA, Part P. Determination of cadmium and zinc in Alzheimer's brain tissue using inductively coupled plasma mass spectrometry. J Neurol Sci 2002;195:1-10.
75. Samudralwar DL, Diprete CC, Ni BF, Ehmann WD, Markesbery WR. Elemental imbalances in the olfactory pathway in Alzheimer's disease. J Neurol Sci 1995;130:139-145.
76. Deibel MA, Ehmann WD, Markesbery WR. Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress. J Neurol Sci 1996;143:137-142.
77. Religa D, Strozyk D, Cherny RA, et al. Elevated cortical zinc in Alzheimer disease. Neurology 2006;67:69-75.
78. Schrag M, Crofton A, Zabel M, et al. Effect of cerebral amyloid angiopathy on brain iron, copper, and zinc in Alzheimer's disease. J Alzheimers Dis 2011;24:137-149.
79. Suh SW, Jensen KB, Jensen MS, et al. Histochemically-reactive zinc in amyloid plaques, angiopathy, and degenerating neurons of Alzheimer's diseased brains. Brain Res 2000;852:274-278.
80. Stoltenberg M, Bruhn M, Sondergaard C, et al. Immersion autometallographic tracing of zinc ions in Alzheimer beta-amyloid plaques. Histochem Cell Biol 2005;123:605-611.
81. Miller LM, Wang Q, Telivala TP, Smith RJ, Lanzirotti A, Miklossy J. Synchrotron-based infrared and X-ray imaging shows focalized accumulation of Cu and Zn co-localized with beta-amyloid deposits in Alzheimer's disease. J Struct Biol 2006;155:30-37.
82. Lovell MA, Robertson JD, Teesdale WJ, Campbell JL, Markesbery WR. Copper, iron and zinc in Alzheimer's disease senile plaques. J Neurol Sci 1998;158:47-52.
83. Bush AI, Multhaup G, Moir RD, et al. A novel zinc(II) binding site modulates the function of the beta A4 amyloid protein precursor of Alzheimer's disease. J Biol Chem 1993;268:16109-16112.
84. Lee JY, Cole TB, Palmiter RD, Suh SW, Koh JY. Contribution by synaptic zinc to the gender-disparate plaque formation in human Swedish mutant APP transgenic mice. Proc Natl Acad Sci U S A 2002;99:7705-7710.
85. Adlard PA, Parncutt JM, Finkelstein DI, Bush AI. Cognitive loss in zinc transporter-3 knock-out mice: a phenocopy for the synaptic and memory deficits of Alzheimer's disease? J Neurosci 2010;30:1631-1636.
86. Lammich S, Kojro E, Postina R, et al. Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. Proc Natl Acad Sci U S A 1999;96:3922-3927.
87. Hoke DE, Tan JL, Ilaya NT, et al. In vitro gamma-secretase cleavage of the Alzheimer's amyloid precursor protein correlates to a subset of presenilin complexes and is inhibited by zinc. FEBS J 2005;272:5544-5557.
88. Greenough MA, Volitaskis I, Li Q-X, et al. Presenilins promote the cellular uptake of copper and zinc and maintain copper chaperone of SOD1-dependent copper/zinc superoxide dismutase activity. J Biol Chem 2011;286:9776-9786.
89. Bush AI, Pettingell WH, Paradis MD, Tanzi RE. Modulation of A beta adhesiveness and secretase site cleavage by zinc. J Biol Chem 1994;269:12152-12158.
90. Crouch PJ, Tew DJ, Du T, et al. Restored degradation of the Alzheimer's amyloid-beta peptide by targeting amyloid formation. J Neurochem 2009;108:1198-1207.
91. Boom A, Authelet M, Dedecker R, et al. Bimodal modulation of tau protein phosphorylation and conformation by extracellular Zn2+ in human-tau transfected cells. Biochim Biophys Acta 2009;1793:1058-1067.
92. Mo Z-Y, Zhu Y-Z, Zhu H-L, Fan J-B, Chen J, Liang Y. Low micromolar zinc accelerates the fibrillization of human tau via bridging of Cys-291 and Cys-322. J Biol Chem 2009;284:34648-34657.
93. Huang Y, Wu Z, Cao Y, Lang M, Lu B, Zhou B. Zinc binding directly regulates tau toxicity independent of tau hyperphosphorylation. Cell Rep 2014;8:831-842.
94. An W-L, Bjorkdahl C, Liu R, Cowburn RF, Winblad B, Pei J-J. Mechanism of zinc-induced phosphorylation of p70 S6 kinase and

glycogen synthase kinase 3beta in SH-SY5Y neuroblastoma cells. J Neurochem 2005;92:1104-1115.
95. Pei J-J, An W-L, Zhou X-W, et al. P70 S6 kinase mediates tau phosphorylation and synthesis. FEBS Lett 2006;580:107-114.
96. Lei P, Ayton S, Bush AI, Adlard PA. GSK-3 in neurodegenerative diseases. Int J Alz Dis 2011;2011:189246.
97. Ayton S, Lei P. Nigral iron elevation is an invariable feature of Parkinson's disease and is a sufficient cause of neurodegeneration. Biomed Research Int 2014;2014:581256.
98. Ayton S, Lei P, Adlard PA, et al. Iron accumulation confers neurotoxicity to a vulnerable population of nigral neurons: implications for Parkinson's disease. Mol Neurodegener 2014;9:27.
99. Goodman L. Alzheimer's disease; a clinico-pathologic analysis of twenty-three cases with a theory on pathogenesis. J Nerv Mental Dis 1953;118:97-130.
100. Connor JR, Menzies SL, St Martin SM, Mufson EJ. A histochemical study of iron, transferrin, and ferritin in Alzheimer's diseased brains. J Neurosci Res 1992;31:75-83.
101. Connor JR, Snyder BS, Beard JL, Fine RE, Mufson EJ. Regional distribution of iron and iron-regulatory proteins in the brain in aging and Alzheimer's disease. J Neurosci Res 1992;31:327-335.
102. Collingwood JF, Mikhaylova A, Davidson M, et al. In situ characterization and mapping of iron compounds in Alzheimer's disease tissue. J Alzheimers Dis 2005;7:267-272.
103. Baltes C, Princz-Kranz F, Rudin M, Mueggler T. Detecting amyloid-beta plaques in Alzheimer's disease. Methods Mol Biol 2011;711:511-533.
104. Meadowcroft MD, Connor JR, Smith MB, Yang QX. MRI and histological analysis of beta-amyloid plaques in both human Alzheimer's disease and APP/PS1 transgenic mice. J Magn Reson Imaging 2009;29:997-1007.
105. Collingwood JF, Chong RK, Kasama T, et al. Three-dimensional tomographic imaging and characterization of iron compounds within Alzheimer's plaque core material. J Alzheimers Dis 2008;14:235-245.
106. Duce JA, Tsatsanis A, Cater MA, et al. Iron-export ferroxidase activity of β-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. Cell 2010;142:857-867.
107. Andrasi E, Farkas E, Scheibler H, Reffy A, Bezur L. Al, Zn, Cu, Mn and Fe levels in brain in Alzheimer's disease. Arch Gerontol Geriatr 1995;21:89-97.
108. Smith MA, Harris PL, Sayre LM, Perry G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. Proc Natl Acad Sci U S A 1997;94:9866-9868.
109. Hare D, Ayton S, Bush A, Lei P. A delicate balance: Iron metabolism and diseases of the brain. Front Aging Neurosci 2013;5:34.
110. Salvador GA, Uranga RM, Giusto NM. Iron and mechanisms of neurotoxicity. Int J Alz Dis 2010;2011:720658.
111. Mark RJ, Hensley K, Butterfield DA, Mattson MP. Amyloid beta-peptide impairs ion-motive ATPase activities: evidence for a role in loss of neuronal Ca2+ homeostasis and cell death. J Neurosci 1995;15:6239-6249.
112. Ahuja RP, Borchman D, Dean WL, et al. Effect of oxidation on Ca2+ -ATPase activity and membrane lipids in lens epithelial microsomes. Free Radic Biol Med 1999;27:177-185.
113. Moreau VH, Castilho RF, Ferreira ST, Carvalho-Alves PC. Oxidative damage to sarcoplasmic reticulum Ca2+-ATPase AT submicromolar iron concentrations: evidence for metal-catalyzed oxidation. Free Radic Biol Med 1998;25:554-560.
114. Kaplan P, Matejovicova M, Mezesova V. Iron-induced inhibition of Na+, K(+)-ATPase and Na+/Ca2+ exchanger in synaptosomes: protection by the pyridoindole stobadine. Neurochem Res 1997;22:1523-1529.
115. Gnana-Prakasam JP, Thangaraju M, Liu K, et al. Absence of iron-regulatory protein Hfe results in hyperproliferation of retinal pigment epithelium: role of cystine/glutamate exchanger. Biochem J 2009;424:243-252.
116. Yu J, Guo Y, Sun M, Li B, Zhang Y, Li C. Iron is a potential key mediator of glutamate excitotoxicity in spinal cord motor neurons. Brain Res 2009;1257:102-107.
117. Mitchell RM, Lee SY, Simmons Z, Connor JR. HFE polymorphisms affect cellular glutamate regulation. Neurobiol Aging 2011;32:1114-1123.
118. Pedersen WA, Fu W, Keller JN, et al. Protein modification by the lipid peroxidation product 4-hydroxynonenal in the spinal cords of amyotrophic lateral sclerosis patients. Ann Neurol 1998;44:819-824.
119. Pedersen WA, Chan SL, Mattson MP. A mechanism for the neuroprotective effect of apolipoprotein E: isoform-specific modification by the lipid peroxidation product 4-hydroxynonenal. J Neurochem 2000;74:1426-1433.
120. Keller JN, Mark RJ, Bruce AJ, et al. 4-Hydroxynonenal, an aldehydic product of membrane lipid peroxidation, impairs glutamate transport and mitochondrial function in synaptosomes. Neuroscience 1997;80:685-696.
121. Strugatsky D, Gottschalk KE, Goldshleger R, Bibi E, Karlish SJ. Expression of Na+, K+-ATPase in *Pichia pastoris*: analysis of wild type and D369N mutant proteins by Fe2+-catalyzed oxidative cleavage and molecular modeling. J Biol Chem 2003;278:46064–46073.
122. Munoz P, Humeres A, Elgueta C, Kirkwood A, Hidalgo C, Nunez MT. Iron mediates N-methyl-D-aspartate receptor-dependent stimulation of calcium-induced pathways and hippocampal synaptic plasticity. J Biol Chem 2011;286:13382-13392.
123. Jiang X, Mu D, Biran V, et al. Activated Src kinases interact with the N-methyl-D-aspartate receptor after neonatal brain ischemia. Ann Neurol 2008;63:632-641.
124. Nakamichi N, Ohno H, Nakamura Y, Hirai T, Kuramoto N, Yoneda Y. Blockade by ferrous iron of Ca2+ influx through N-methyl-D-aspartate receptor channels in immature cultured rat cortical neurons. J Neurochem 2002;83:1-11.
125. Shinkyo R, Guengerich FP. Cytochrome P450 7A1 cholesterol 7alpha-hydroxylation: individual reaction steps in the catalytic cycle and rate-limiting ferric iron reduction. J Biol Chem 2011;286:4632-4643.
126. Graham RM, Chua AC, Carter KW, et al. Hepatic iron loading in mice increases cholesterol biosynthesis. Hepatology 2010;52:462-471.
127. Kraml PJ, Klein RL, Huang Y, Nareika A, Lopes-Virella MF. Iron loading increases cholesterol accumulation and macrophage scavenger receptor I expression in THP-1 mononuclear phagocytes. Metabolism 2005;54:453-459.
128. Francis N, Wong SH, Hampson P, et al. Lactoferrin inhibits neutrophil apoptosis via blockade of proximal apoptotic signaling events. Biochim Biophys Acta 2011;1813:1822-1826.
129. Yurkova I, Kisel M, Arnhold J, Shadyro O. Iron-mediated free-radical formation of signaling lipids in a model system. Chem Phys Lipids 2005;137:29-37.
130. Yehuda S, Rabinovitz-Shenkar S, Carasso RL. Effects of essential fatty acids in iron deficient and sleep-disturbed attention deficit hyperactivity disorder (ADHD) children. Eur J Clin Nutr 2011;65:1167-1169.
131. LeBlanc CP, Fiset S, Surette ME, Turgeon O'Brien H, Rioux FM. Maternal iron deficiency alters essential fatty acid and eicosanoid metabolism and increases locomotion in adult guinea pig offspring. J Nutr 2009;139:1653-1659.
132. Brown KE, Dennery PA, Ridnour LA, et al. Effect of iron overload and dietary fat on indices of oxidative stress and hepatic fibrogenesis in rats. Liver Int 2003;23:232-242.
133. Dougherty JJ, Croft WA, Hoekstra WG. Effects of ferrous chloride and iron dextran on lipid peroxidation in vivo in vitamin E and selenium adequate and deficient rats. J Nutr 1981;111:1784-1796.
134. Isaac G, Fredriksson A, Danielsson R, Eriksson P, Bergquist J. Brain lipid composition in postnatal iron-induced motor behavior

alterations following chronic neuroleptic administration in mice. FEBS J 2006;273:2232-2243.
135. Jenkins KJ, Kramer JK. Effect of excess dietary iron on lipid composition of calf liver, heart, and skeletal muscle. J Dairy Sci 1988;71:435-441.
136. Mattson MP. Metal-catalyzed disruption of membrane protein and lipid signaling in the pathogenesis of neurodegenerative disorders. Ann N Y Acad Sci 2004;1012:37-50.
137. Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 2012;149:1060-1072.
138. Yagoda N, von Rechenberg M, Zaganjor E, et al. RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. Nature 2007;447:864-868.
139. Yang WS, Stockwell BR. Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells. Chem Biol 2008;15:234-245.
140. Rogers JT, Randall JD, Cahill CM, et al. An iron-responsive element type II in the 5′-untranslated region of the Alzheimer's amyloid precursor protein transcript. J Biol Chem 2002;277:45518-45528.
141. Li X, Liu Y, Zheng Q, et al. Ferritin light chain interacts with PEN-2 and affects gamma-secretase activity. Neurosci Lett 2013;548:90-94.
142. Becerril-Ortega J, Bordji K, Freret T, Rush T, Buisson A. Iron overload accelerates neuronal amyloid-beta production and cognitive impairment in transgenic mice model of Alzheimer's disease. Neurobiol Aging 2014;35:2288-2301.
143. Mantyh PW, Ghilardi JR, Rogers S, et al. Aluminum, iron, and zinc ions promote aggregation of physiological concentrations of beta-amyloid peptide. J Neurochem 1993;61:1171-1174.
144. Huang X, Atwood CS, Moir RD, Hartshorn MA, Tanzi RE, Bush AI. Trace metal contamination initiates the apparent auto-aggregation, amyloidosis, and oligomerization of Alzheimer's Abeta peptides. J Biol Inorg Chem 2004;9:954-960.
145. Schubert D, Chevion M. The role of iron in beta amyloid toxicity. Biochem Biophys Res Commun 1995;216:702-707.
146. Liu B, Moloney A, Meehan S, et al. Iron promotes the toxicity of amyloid beta peptide by impeding its ordered aggregation. J Biol Chem 2011;286:4248-4256.
147. Rottkamp CA, Raina AK, Zhu XW, et al. Redox-active iron mediates amyloid-beta toxicity. Free Radic Biol Med 2001;30:447-450.
148. Rival T, Page RM, Chandraratna DS, et al. Fenton chemistry and oxidative stress mediate the toxicity of the beta-amyloid peptide in a *Drosophila* model of Alzheimer's disease. Eur J Neurosci 2009;29:1335-1347.
149. Kuperstein F, Yavin E. Pro-apoptotic signaling in neuronal cells following iron and amyloid beta peptide neurotoxicity. J Neurochem 2003;86:114-125.
150. García de Ancos J, Correas I, Avila J. Differences in microtubule binding and self-association abilities of bovine brain tau isoforms. J Biol Chem 1993;268:7976-7982.
151. Ledesma MD, Avila J, Correas I. Isolation of a phosphorylated soluble tau fraction from Alzheimer's disease brain. Neurobiol Aging 1995;16:515-522.
152. Yamamoto A, Shin R-W, Hasegawa K, et al. Iron (III) induces aggregation of hyperphosphorylated tau and its reduction to iron (II) reverses the aggregation: implications in the formation of neurofibrillary tangles of Alzheimer's disease. J Neurochem 2002;82:1137-1147.
153. Lovell MA, Xiong S, Xie C, Davies PL, Markesbery WR. Induction of hyperphosphorylated tau in primary rat cortical neuron cultures mediated by oxidative stress and glycogen synthase kinase-3. J Alzheimers Dis 2004;6:659-671.
154. Chan A, Shea TB. Dietary and genetically-induced oxidative stress alter tau phosphorylation: influence of folate and apolipoprotein E deficiency. J Alzheimers Dis 2006;9:399-405.
155. Huang X, Dai J, Huang C, Zhang Q, Bhanot O, Pelle E. Deferoxamine synergistically enhances iron-mediated AP-1 activation: a showcase of the interplay between extracellular-signal-regulated kinase and tyrosine phosphatase. Free Radic Res 2007;41:1135-1142.
156. Muñoz P, Zavala G, Castillo K, Aguirre P, Hidalgo C, Nuñez MT. Effect of iron on the activation of the MAPK/ERK pathway in PC12 neuroblastoma cells. Biol Res 2006;39:189-190.
157. Ksiezak-Reding H, Binder LI, Yen S-HC. Immunochemical and biochemical characterization of tau proteins in normal and Alzheimer's disease brains with Alz 50 and Tau-1. J Biol Chem 1988;263:7948-7953.
158. Khatoon S, Grundke-Iqbal I, Iqbal K. Levels of normal and abnormally phosphorylated tau in different cellular and regional compartments of Alzheimer disease and control brains. FEBS Lett 1994;351:80-84.
159. Zhukareva V, Sundarraj S, Mann D, et al. Selective reduction of soluble tau proteins in sporadic and familial frontotemporal dementias: an international follow-up study. Acta Neuropathol 2003;105:469-476.
160. van Eersel J, Bi M, Ke YD, et al. Phosphorylation of soluble tau differs in Pick's disease and Alzheimer's disease brains. J Neural Transm 2009;116:1243-1251.
161. McCarthy RC, Park YH, Kosman DJ. sAPP modulates iron efflux from brain microvascular endothelial cells by stabilizing the ferrous iron exporter ferroportin. EMBO Rep 2014;15:809-815.
162. Ayton S, Zhang M, Roberts BR, et al. Ceruloplasmin and beta-amyloid precursor protein confer neuroprotection in traumatic brain injury and lower neuronal iron. Free Radic Biol Med 2014;69:331-337.
163. Madsen E, Gitlin JD. Copper and iron disorders of the brain. Annu Rev Neurosci 2007;30:317-337.
164. Barnham KJ, Masters CL, Bush AI. Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov 2004;3:205-214.
165. Barnham KJ, Ciccotosto GD, Tickler AK, et al. Neurotoxic, redox-competent Alzheimer's beta-amyloid is released from lipid membrane by methionine oxidation. J Biol Chem 2003;278:42959-42965.
166. Puglielli L, Friedlich AL, Setchell KDR, et al. Alzheimer disease beta-amyloid activity mimics cholesterol oxidase. J Clin Invest 2005;115:2556-2563.
167. Robinson NJ, Winge DR. Copper metallochaperones. Annu Rev Biochem 2010;79:537-562.
168. Ayton S, Lei P, Duce JA, et al. Ceruloplasmin dysfunction and therapeutic potential for Parkinson disease. Ann Neurol 2013;73:554-559.
169. Wong BX, Ayton S, Lam LQ, et al. A comparison of ceruloplasmin to biological polyanions in promoting the oxidation of Fe under physiologically relevant conditions. Biochim Biophys Acta 2014;1840:3299-3310.
170. Harris ZL, Takahashi Y, Miyajima H, Serizawa M, MacGillivray RT, Gitlin JD. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. Proc Natl Acad Sci U S A 1995;92:2539-2543.
171. Talmard C, Guilloreau L, Coppel Y, Mazarguil H, Faller P. Amyloid-beta peptide forms monomeric complexes with Cu(II) and Zn(II) prior to aggregation. Chembiochem 2007;8:163-165.
172. Atwood CS, Moir RD, Huang X, et al. Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. J Biol Chem 1998;273:12817-12826.
173. Jiao Y, Han DX, Yang P. Molecular modeling of the inhibitory mechanism of copper(II) on aggregation of amyloid beta-peptide. Sci China Ser B 2005;48:580-590.

174. Jiao Y, Yang P. Mechanism of copper(II) inhibiting Alzheimer's amyloid beta-peptide from aggregation: a molecular dynamics investigation. J Phys Chem B 2007;111:7646-7655.
175. Tõugu V, Karafin A, Zovo K, et al. Zn(II)- and Cu(II)-induced non-fibrillar aggregates of amyloid-beta (1-42) peptide are transformed to amyloid fibrils, both spontaneously and under the influence of metal chelators. J Neurochem 2009;110:1784-1795.
176. Wu W-h, Lei P, Liu Q, et al. Sequestration of copper from beta-amyloid promotes selective lysis by cyclen-hybrid cleavage agents. J Biol Chem 2008;283:31657-31664.
177. Perrone L, Mothes E, Vignes M, et al. Copper transfer from Cu-Abeta to human serum albumin inhibits aggregation, radical production and reduces Abeta toxicity. Chembiochem 2010;11:110-118.
178. Huang X, Atwood CS, Hartshorn MA, et al. The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. Biochemistry 1999;38:7609-7616.
179. Huang X, Cuajungco MP, Atwood CS, et al. Cu(II) potentiation of alzheimer abeta neurotoxicity. Correlation with cell-free hydrogen peroxide production and metal reduction. J Biol Chem 1999;274:37111-37116.
180. Jiang D, Men L, Wang J, et al. Redox reactions of copper complexes formed with different beta-amyloid peptides and their neuropathological relevance. Biochemistry 2007;46:9270-9282.
181. Butterfield DA, Pocernich CB. The glutamatergic system and Alzheimer's disease: therapeutic implications. CNS Drugs 2003;17:641-652.
182. Francis PT, Sims NR, Procter AW, Bowen DM. Cortical pyramidal neurone loss may cause glutamatergic hypoactivity and cognitive impairment in Alzheimer's disease: investigative and therapeutic perspectives. J Neurochem 1993;60:1589-1604.
183. Hopt A, Korte S, Fink H, et al. Methods for studying synaptosomal copper release. J Neurosci Methods 2003;128:159-172.
184. Hartter DE, Barnea A. Evidence for release of copper in the brain: depolarization-induced release of newly taken-up 67copper. Synapse 1988;2:412-415.
185. Schlief ML, Craig AM, Gitlin JD. NMDA receptor activation mediates copper homeostasis in hippocampal neurons. J Neurosci 2005;25:239-246.
186. Schlief ML, West T, Craig AM, Holtzman DM, Gitlin JD. Role of the Menkes copper-transporting ATPase in NMDA receptor-mediated neuronal toxicity. Proc Natl Acad Sci U S A 2006;103:14919-14924.
187. Soragni A, Zambelli B, Mukrasch MD, et al. Structural characterization of binding of Cu(II) to tau protein. Biochemistry 2008;47:10841-10851.
188. Ma QF, Li YM, Du JT, et al. Binding of copper (II) ion to an Alzheimer's tau peptide as revealed by MALDI-TOF MS, CD, and NMR. Biopolymers 2005;79:74-85.
189. Ma Q, Li Y, Du J, et al. Copper binding properties of a tau peptide associated with Alzheimer's disease studied by CD, NMR, and MALDI-TOF MS. Peptides 2006;27:841-89.
190. Zhou LX, Du JT, Zeng ZY, et al. Copper (II) modulates in vitro aggregation of a tau peptide. Peptides 2007;28:2229-2234.
191. Su XY, Wu WH, Huang ZP, et al. Hydrogen peroxide can be generated by tau in the presence of Cu(II). Biochem Biophys Res Commun 2007;358:661-665.
192. Sayre LM, Perry G, Harris PL, Liu Y, Schubert KA, Smith MA. In situ oxidative catalysis by neurofibrillary tangles and senile plaques in Alzheimer's disease: a central role for bound transition metals. J Neurochem 2000;74:270-279.
193. Kitazawa M, Cheng D, Laferla FM. Chronic copper exposure exacerbates both amyloid and tau pathology and selectively dysregulates cdk5 in a mouse model of AD. J Neurochem 2009;108:1550-1560.
194. Crouch PJ, Hung LW, Adlard PA, et al. Increasing Cu bioavailability inhibits Abeta oligomers and tau phosphorylation. Proc Natl Acad Sci U S A 2009;106:381-386.
195. Tateishi J. Subacute myelo-optico-neuropathy: clioquinol intoxication in humans and animals. Neuropathology 2000;20(Suppl.):S20–S24.
196. Cherny RA, Atwood CS, Xilinas ME, et al. Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron 2001;30:665-676.
197. Di Vaira M, Bazzicalupi C, Orioli P, Messori L, Bruni B, Zatta P. Clioquinol, a drug for Alzheimer's disease specifically interfering with brain metal metabolism: structural characterization of its zinc(II) and copper(II) complexes. Inorg Chem 2004;43:3795-3797.
198. Treiber C, Simons A, Strauss M, et al. Clioquinol mediates copper uptake and counteracts copper efflux activities of the amyloid precursor protein of Alzheimer's disease. Biol Chem 2004;279:51958-51964.
199. Raman B, Ban T, Yamaguchi K-i, et al. Metal ion-dependent effects of Clioquinol on the fibril growth of an amyloid {beta} peptide. J Biol Chem 2005;280:16157-16162.
200. Priel T, Aricha-Tamir B, Sekler I. Clioquinol attenuates zinc-dependent beta-cell death and the onset of insulitis and hyperglycemia associated with experimental type I diabetes in mice. Eur J Pharmacol 2007;565:232-239.
201. Barrea RA, Chen D, Irving TC, Dou QP. Synchrotron X-ray imaging reveals a correlation of tumor copper speciation with Clioquinol's anticancer activity. J Cell Biochem 2009;108:96-105.
202. Nitzan YB, Sekler I, Frederickson CJ, et al. Clioquinol effects on tissue chelatable zinc in mice. J Mol Med (Berl) 2003;81:637-644.
203. Adlard PA, Cherny RA, Finkelstein DI, et al. Rapid restoration of cognition in Alzheimer's transgenic mice with 8-hydroxy quinoline analogs is associated with decreased interstitial Abeta. Neuron 2008;59:43-55.
204. Li C, Wang J, Zhou B. The metal chelating and chaperoning effects of clioquinol: insights from yeast studies. J Alzheimers Dis 2010;21:1249-1262.
205. Park MH, Lee SJ, Byun HR, et al. Clioquinol induces autophagy in cultured astrocytes and neurons by acting as a zinc ionophore. Neurobiol Dis 2011;42:242-251.
206. Crouch PJ, Savva MS, Hung LW, et al. The Alzheimer's therapeutic PBT2 promotes amyloid-beta degradation and GSK3 phosphorylation via a metal chaperone activity. J Neurochem 2011;119:220-230.
207. Atamna H, Frey WH. A role for heme in Alzheimer's disease: heme binds amyloid beta and has altered metabolism. Proc Natl Acad Sci U S A 2004;101:11153-11158.
208. Choi SM, Choi KO, Park YK, Cho H, Yang EG, Park H. Clioquinol, a Cu(II)/Zn(II) chelator, inhibits both ubiquitination and asparagine hydroxylation of hypoxia-inducible factor-1alpha, leading to expression of vascular endothelial growth factor and erythropoietin in normoxic cells. J Biol Chem 2006;281:34056-34063.
209. Felkai S, Ewbank JJ, Lemieux J, Labbe JC, Brown GG, Hekimi S. CLK-1 controls respiration, behavior and aging in the nematode *Caenorhabditis elegans*. EMBO J 1999;18:1783-1792.
210. Kaur D, Yantiri F, Rajagopalan S, et al. Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: a novel therapy for Parkinson's disease. Neuron 2003;37:899-909.
211. LeVine H, 3rd, Ding Q, Walker JA, Voss RS, Augelli-Szafran CE. Clioquinol and other hydroxyquinoline derivatives inhibit Abeta(1-42) oligomer assembly. Neurosci Lett 2009;465:99-103.
212. Mancino AM, Hindo SS, Kochi A, Lim MH. Effects of clioquinol on metal-triggered amyloid-beta aggregation revisited. Inorg Chem 2009;48:9596-9598.
213. Stoppelkamp S, Bell HS, Palacios-Filardo J, Shewan DA, Riedel G, Platt B. In vitro modelling of Alzheimer's disease: degeneration and cell death induced by viral delivery of amyloid and tau. Exp Neurol 2011;229:226-237.
214. Grossi C, Francese S, Casini A, et al. Clioquinol decreases amyloid-beta burden and reduces working memory impairment in a

transgenic mouse model of Alzheimer's disease. J Alzheimers Dis 2009;17:423-440.
215. Ritchie CW, Bush AI, Mackinnon A, et al. Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. Arch Neurol 2003;60:1685-1691.
216. Lannfelt L, Blennow K, Zetterberg H, et al. Safety, efficacy, and biomarker findings of PBT2 in targeting Abeta as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial. Lancet Neurol 2008;7:779-786.
217. Faux NG, Ritchie CW, Gunn AP, et al. PBT2 rapidly improves cognition in Alzheimer's Disease: additional phase II analyses. J Alzheimers Dis 2010;20:509-516.
218. Cherny RA, Ayton S, Finkelstein DI, Bush AI, McColl G, Massa SM. PBT2 reduces toxicity in a *C. elegans* model of polyQ aggregation and extends lifespan, reduces striatal atrophy and improves motor performance in the R6/2 mouse model of Huntington's disease. J Huntingtons Dis 2012;1:211-219.
219. Donnelly PS, Caragounis A, Du T, et al. Selective intracellular release of copper and zinc ions from bis(thiosemicarbazonato) complexes reduces levels of Alzheimer disease amyloid-beta peptide. J Biol Chem 2008;283:4568-4577.
220. Hung LW, Villemagne VL, Cheng L, et al. The hypoxia imaging agent CuII(atsm) is neuroprotective and improves motor and cognitive functions in multiple animal models of Parkinson's disease. J Exp Med 2012;209:837-854.
221. Crapper McLachlan DR, Dalton AJ, Kruck TP, et al. Intramuscular desferrioxamine in patients with Alzheimer's disease. Lancet 1991;337:1304-1308.
222. Devos D, Moreau C, Devedjian JC, et al. Targeting chelatable iron as a therapeutic modality in Parkinson's disease. Antioxid Redox Signal 2014;21:195-210.