

REVIEW

Alzheimer's disease & metals: therapeutic opportunities

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Alzheimer's disease (AD) is the most common age related neurodegenerative disease. Currently, there are no disease modifying drugs, existing therapies only offer short-term symptomatic relief. Two of the pathognomonic indicators of AD are the presence of extracellular protein aggregates consisting primarily of the A β peptide and oxidative stress. Both of these phenomena can potentially be explained by the interactions of A β with metal ions. In addition, metal ions play a pivotal role in synaptic function and their homeostasis is tightly regulated. A breakdown in this metal homeostasis and the generation of toxic A β oligomers are likely to be responsible for the synaptic dysfunction associated with AD. Therefore, approaches that are designed to prevent A β metal interactions, inhibiting the formation of toxic A β species as well as restoring metal homeostasis may have potential as disease modifying strategies for treating AD. This review summarizes the physiological and pathological interactions that metal ions play in synaptic function with particular emphasis placed on interactions with A β . A variety of therapeutic strategies designed to address these pathological processes are also described. The most advanced of these strategies is the so-called 'metal protein attenuating compound' approach, with the lead molecule PBT2 having successfully completed early phase clinical trials. The success of these various strategies suggests that manipulating metal ion interactions offers multiple opportunities to develop disease modifying therapies for AD.

Abbreviations

A β , amyloid- β peptide; AD, Alzheimer's disease; CQ, clioquinol; MPAC, metal protein attenuating compound; ROS, reactive oxygen species

Introduction

Dementia is characterized by the loss of or decline in memory and other cognitive abilities. Alzheimer's disease (AD) (Alzheimer *et al.*, 1995) is the most common type of dementia and accounts for an estimated 60–80% of cases. AD is an irreversible, progressive neurodegenerative disorder leading invariably to death usually within 7–10 years of diagnosis. Age is the dominant risk factor in AD, and the progressive nature of neurodegeneration ultimately leads to synaptic failure and neuronal damage (Masters and Beyreuther, 1988) in cortical areas of the brain essential for memory and higher mental functions. The increase in the number of new cases of AD is the direct consequence of an improvement in life expectancy. There are currently 18 million people worldwide with AD and this figure is projected to nearly double by 2025 to 34 million (Alzheimer's Association, 2010). In the USA alone the esti-

mated annual cost of caring for over 5 million AD patients is \$172 billion (Alzheimer's Association, 2010). Besides the monetary cost, the spouses, relatives and friends of AD patients experience emotional, physical and financial stresses during the years of caregiving that is impossible to quantify. Clearly, AD represents a major socio-economic problem which requires better assessment, management and effective therapies in order to ease the burden of the disease. However, despite an ever increasing knowledge of genetics, epidemiology, risk factors and neuropathological mechanisms, there is still no cure for AD.

The pathognomonic indicators of disease are the presence of senile plaques (Kromer Vogt *et al.*, 1990), neurofibrillary tangles, neurophil threads, amyloid- β peptide (A β) deposition and selective loss of neurons and decreased synaptic density in the post-mortem brain. Deposition of amyloid plaques in AD is most obvious pathological feature; the major constituent of these deposits being the A β peptide that is

proteolytically cleaved from the membrane bound amyloid precursor protein (APP). Genetic evidence from cases of familial AD indicates that A β metabolism is linked to the disease. While the precise function of APP is still debated; evidence suggests it has functions in maintaining metal homeostasis. These include roles in modulating Cu efflux from cells (Bellingham *et al.*, 2004) and acting as a ferroxidase (Duce *et al.*, 2010). This ferroxidase activity of APP is inhibited by Zn.

Over the past 20 years, there has been mounting evidence implicating aberrant metal biochemistry in the pathogenesis of AD. As the data have been accumulated there has also been a growing awareness of the critical role that transition metal ions play in modulating neurotransmission at the glutamatergic synapses in the cortex and hippocampus, processes that are pivotal to properly functioning memory. Therefore, the ability to manipulate metal homeostasis and localization offers the potential for the development of new therapeutic strategies for the treatment of AD. It is this metal biology and therapeutic strategies that is the subject of this review.

Metals and the synapse

Synaptic dysfunction has been implicated as the primary cause of the memory deficits associated with AD (Selkoe, 2002). Cu and Zn have been reported to play key roles in regulating synaptic function. Zn is released in high μ M concentrations from presynaptic glutamatergic nerve terminals into the synaptic cleft upon neuronal activation. This pool of synaptic Zn interacts with a variety of receptors, ion channels and transporters. Zn is co-released into the synapse with the neurotransmitter glutamate. While glutamate is known to stimulate the N-methyl-D-aspartate receptor (NMDAr), Zn inhibits NMDAr in a voltage dependent and voltage independent manner, implicating the metal in a modulatory role (Smart *et al.*, 2004). The zinc transporter protein ZnT3 is primarily responsible for loading Zn into the synaptic vesicles. A recent study examining the effects of knocking out ZnT3 in mice found that these animals had age dependent deficits in learning and memory that were associated with decreased levels of a range of synaptic proteins including SNAP25, PSD25 and NMDAr subunits 2a and b (Adlard *et al.*, 2010). These results lead the authors to suggest that these knockout animals represented a 'phenocopy for the synaptic and memory deficits of Alzheimer's disease'. This statement was further supported by evidence that levels of this protein are significantly decreased in human brain tissue as a consequence of ageing and in AD. Cu also plays a role in regulating synaptic function as it is released from the post-synaptic side of the cleft following NMDA activation, this release is facilitated by trafficking of ATP7a, mutations in which can cause Menkes disease (Schlief *et al.*, 2006a,b).

While metals play a role in a variety of essential biological functions the transport and utilization of these are generally tightly regulated as the aspects of their chemistry that make them physiologically useful can also give rise to pathological phenomena. The classic example of this is the storage, transport and utilization of oxygen, the same chemistry that allows metal-based enzymes to do this is essentially the same chemistry that facilitates the production of toxic reactive

oxygen/nitrogen species that gives rise to oxidative stress. As a result, nature has evolved a complex system of metal transporters and chaperones that tightly regulate metal homeostasis and pools of 'free' metal ions are rare, where in a biological context we mean that 'free' metals are bound by labile low affinity and therefore readily exchangeable ligands. Interestingly, it appears that both the Zn that is released from the presynaptic terminals and Cu that is released from the post-synaptic terminals following synapse stimulation are predominately in a 'free' exchangeable form. In healthy tissue these released metal ions will be retaken up by the cells by metal chaperones such as metallothionein-3.

A β interactions with Cu/Zn

Another molecule that is known to be released into the synapse is A β ; therefore, the synapse is one location where A β and 'free' Cu and Zn are present in sufficiently high quantities to promote an interaction. A β deposition begins within the synapse (Terry, 1996), and the amyloid plaques can be described as metal sinks as remarkably high concentrations of Cu (\sim 400 μ M), Zn (\sim 1 mM) and Fe (\sim 1 mM) have been reported in senile plaques isolated from AD subjects (Lovell *et al.*, 1998). The potential role that metals may play in amyloid plaque formation is highlighted by the finding that age- and female-sex-related plaque formation in Tg2576 transgenic mice was reduced by genetic ablation of the ZnT-3 protein (Lee *et al.*, 2002).

Two of the pathognomonic features of AD; A β deposition and oxidative stress can potentially be attributed to A β 's interactions with metal ions. Synthetic A β will react with both Zn and Cu to form aggregates while A β will react with Cu to generate reactive oxygen species (ROS) (Bush, 2003; Smith *et al.*, 2007). *In vitro* studies utilizing synthetic A β have shown that low μ M levels of Zn²⁺ will induce protease-resistant aggregation and precipitation of A β (Bush *et al.*, 1994; Huang *et al.*, 1997; 1999; 2000). Huang *et al.* (1997) tested the Zn induced A β aggregation for reversibility *in vitro* and found that low concentration of Zn²⁺ (20 nmol) induced protease-resistant (β -sheet) aggregation and precipitation of A β ; this aggregation could be reversed by the use of metal chelators. Similar phenomena were observed with Cu²⁺ and Fe³⁺ at physiological conditions (pH = 7) and the aggregation was exaggerated at acidic pH (Atwood *et al.*, 1998).

A β coordination of Cu leads to the generation of ROS involving the reduction of the oxidation state of the coordinated Cu²⁺ to Cu⁺ and this reduction must be accompanied by the oxidation of another moiety, endogenous molecules such as thiols, ascorbate, lipid or A β side-chains (Butterfield *et al.*, 2001; Turnbull *et al.*, 2001; Barnham *et al.*, 2003a,b; Puglielli *et al.*, 2005). Mass spectrometry has shown that Cu²⁺ ions are able to oxygenate A β at a number of different residues including the histidine residues 6, 13, 14 and methionine 35 (Atwood *et al.*, 2000; Schoneich, 2002; Barnham *et al.*, 2003a). Other redox mediated modifications include aldehyde adducts to the lysine residues (Chen *et al.*, 2007) and tyrosine modified to moieties such as DOPA, dopamine, dopamine quinone, dihydroxyindol and isodityrosine (Ali *et al.*, 2005; 2006). Tyrosine is particularly susceptible to free radical attack due to the conjugated aromatic ring.

Elevated dityrosine and 3-nitrotyrosine within the neuronal lesions in AD brain have been reported. *In vitro* A β 42 in the presence of Cu²⁺ and H₂O₂ forms dityrosine cross-linked oligomers (Barnham *et al.*, 2004; Atwood *et al.*, 2004), a modification that is resistant to proteolysis. The formation of dityrosine linkage in A β facilitates further peptide aggregation, leading to the formation of higher order oligomers.

A β toxicity

A variety of hypotheses have been proposed for the mechanism by which A β induces its neurotoxic effects, the one point of consensus most hypotheses have is the requirement for A β to aggregate. Nearly every conceivable aggregate from dimer to fully formed fibrillar structures have been reported as toxic. In general, the various aggregates have been very poorly characterized, though there is a growing interest in soluble oligomers, which appear to be particularly toxic (Lesne *et al.*, 2006). It is possible that covalent cross-linking of A β (e.g. dityrosine formation generated by Cu oxidation) contributes to the formation of these toxic soluble species (Barnham *et al.*, 2004). Cu has been shown to potentiate the toxicity of A β to neuronal cell cultures (Atwood *et al.*, 1999; Duce *et al.*, 2006; Fodero-Tavoletti *et al.*, 2007). Zn induced A β oligomers have been shown to elicit toxic responses in hippocampal brain slices via interactions with the NMDAr (Deshpande *et al.*, 2009). The detailed mechanisms of A β toxicity have recently been described elsewhere (Cappai and Barnham, 2008).

Cu/Zn binding sites on A β

As the metal ions Cu and Zn are able to modulate A β structure and function there has been much interest in delineating the nature of the metal coordination site. A β possesses histidine residues at positions 6, 13 and 14, which form a structural element that enables A β to coordinate transition metal ions. A variety of spectroscopic studies have confirmed that the histidine residues constitute the principal site(s) of metal coordination. However, many of these studies have given contradictory results and failed to define a definitive structure for the metal binding site(s). Drew *et al.* (2009a,b) were able to explain these apparent contradictions in the literature using a combination of multifrequency electron paramagnetic resonance and site specific ¹⁵N- and ¹³C-labelling which indicated that there was not a single metal binding but rather an interconverting ensemble of structures. This concept of the Cu coordination site being pleomorphic was further validated by the results from the Faller group (Dorlet *et al.*, 2009; Hureau *et al.*, 2009).

The difficulties in defining the metal binding site(s) on A β have been mirrored by the various attempts to define the metal binding affinity. As A β metal interactions lead to peptide aggregation and precipitation this complicates attempts to define thermodynamic parameters for these interactions. As a result, a variety of binding constants for A β /metal interactions have been published, with K_ds ranging from high μ M to aM. The experimental difficulties associated with obtaining accurate thermodynamic data have recently reviewed (Xiao *et al.*,

2010). The current best estimates for the binding affinity for A β with Zn is in low μ M range while for Cu it is in the sub nM range (Faller, 2009). A more detailed review of the various spectroscopic characterizations of the A β metal binding site and critical appraisal the determination of associated binding affinities has recently been published (Faller, 2009).

Therapeutic strategies

Current medications (United States Food and Drug Administration approved) that have been developed for the treatment of AD include inhibitors of acetylcholinesterase activity (Birks *et al.*, 2006) and antagonists of NMDAr (Areosa *et al.*, 2005). At best these drugs are able to produce modest symptomatic improvements in some patients; they do not cure or stop disease progression (Takeda *et al.*, 2006). As a result, there is a need to search for new therapeutic strategies that target the underlying pathogenic mechanisms in AD.

The majority of attempts to develop disease modifying drugs for AD have centred on reducing levels of A β in the brain. This can be achieved by either lowering the levels of A β production by inhibiting the processing of APP by either beta-amyloid converting enzyme or γ -secretase or increasing clearance primarily through the use of immunization. To date, both approaches have had toxicity issues hinder development. For example, recently a large scale clinical trial of the Eli Lilly's γ -secretase inhibitor semagacestat had to be halted due to the increased risk of skin cancer and worsening cognitive performance (Exance, 2010). Similarly, the first active immunotherapy trial targeting A β had to be halted due to a number of patients developing meningoencephalitis (Orgogozo *et al.*, 2003).

Metal protein attenuating compounds for AD

One approach to developing potential disease modifying drugs for AD is to inhibit the interactions between A β and metals that drive the pathological features of AD such as amyloid deposition and oxidative stress. Broadly speaking, there are two potential classes of molecule capable of preventing such interactions. The first involves inhibiting A β /metal interactions by selectively occupying the metal binding site on A β , thus preventing metal coordination. The initial metal binding site constitutes His-6, His-13 and His-14. However, these residues are natively unstructured in the absence of metal ions, and as a result the design of classic 'lock and key' inhibitors is problematic. However, alternative metal based approaches will be discussed later in the review. A second pharmacological approach is to identify a class of molecules that will effectively compete with the peptide for the metal ions. Metal chelators have been used clinically for decades to target metal overload diseases such as haemochromatosis (Fe) and Wilson's disease (Cu). The chelators used to treat these diseases such as desferrioxime, penicillamine and trientine are intended to deplete metal from overloaded tissue and facilitate its excretion. As such these chelators have very high metal binding affinities and are hydrophilic. These types of

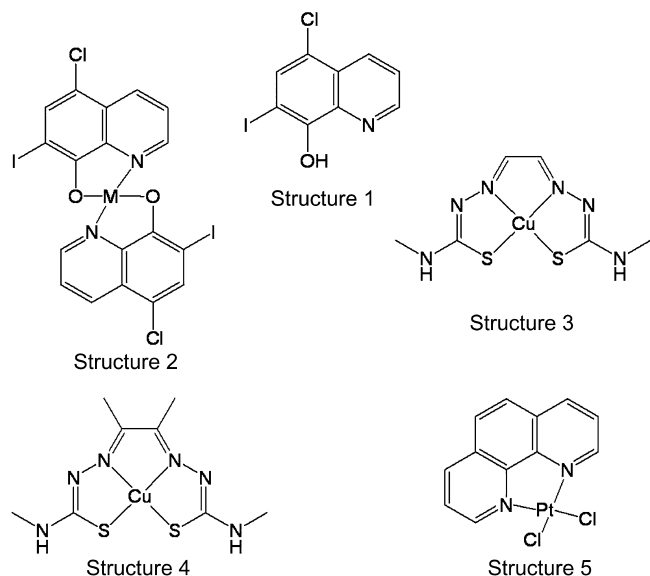


Figure 1

Representative examples of the molecular structures of compounds discussed within this review. Structure 1 is clioquinol (CQ, 5-chloro-7-iodo-8-hydroxyquinoline), the prototypic metal protein attenuating compound (MPAC). Structure 2 is a metal complex of CQ ($M = \text{Cu}$ or Zn), which illustrates that the MPAC metal complexes are neutral hydrophobic molecules capable of delivering metals across cellular membranes. Structures 3 and 4 are examples of the $M(\text{bis}(\text{thiosemicarbazone}))$ complexes. Structure 3 is glyoxalbis($N(4)$ -methyl-3-thiosemicarbazonato) copper(II) ($\text{Cu}^{\text{II}}(\text{gtms})$). This is a neutral metal complex capable of crossing cellular membranes. In an extracellular environment this complex is very stable; however, intracellularly the oxidation state of the metal is reduced from Cu^{2+} to Cu^{+} , which destabilizes the complex and the metal is made bioavailable. Structure 4 is diacetylbis($N(4)$ -methyl-3-thiosemicarbazonato) copper(II) ($\text{Cu}^{\text{II}}(\text{atms})$). The two additional methyl groups sufficiently alter the redox potential of the metal so there is no intracellular reduction and the complex remains intact (i.e. the metal does not become bioavailable). Structure 5 $\text{Pt}^{\text{II}}(1,10\text{-phenanthroline})\text{Cl}_2$ is a representative example of the L-PtCl_2 compounds that are designed to coordinate to $\text{A}\beta$ and inhibit its toxic interactions.

chelators are inappropriate as potential therapeutics for AD. Firstly, the drug has to cross the blood brain barrier and this generally requires small hydrophobic molecules. Secondly, while there are some reports of increases in metal levels in the AD brain, there are equally as many reports that dispute this. As such AD could not be described as a metal overload disease, but perhaps is better described as one where metal homeostasis is perturbed resulting in miss location of metal ions. As these metals are involved in essential neurological functions just removing them with high affinity chelators is potentially problematic.

One goal is to develop compounds that target this mislocated metal bound to $\text{A}\beta$ and restore it to where it came from, that is, within the synapse. To differentiate this approach from the high affinity metal depleting chelators traditionally used in medicine this approach has been labelled metal protein attenuating compounds (MPACs). The prototypic MPAC is clioquinol (CQ, 5-chloro-7-iodo-8-hydroxyquinoline) (Figure 1), a small hydrophobic molecule

that has moderate affinity for metal ions and is capable of crossing the blood-brain barrier. *In vitro* studies (Cherny *et al.*, 2001) showed that CQ was able to inhibit metal mediated aggregation and ROS production by $\text{A}\beta$. CQ was then given orally in a blinded study to Tg2576 transgenic mice, the results showed a 49% decrease in brain $\text{A}\beta$ burden compared with non-treated controls after 9 weeks of treatment. Moreover, treatment did not lead to a systematic decrease in metal levels, which is most probably due to the drug's moderate binding affinities (Cherny *et al.*, 2001). The success of CQ in the transgenic mouse trials encouraged the testing of this molecule in clinical trials. The effect of oral CQ treatment in a randomized, double-blind, placebo-controlled pilot Phase II clinical trial (Ritchie *et al.*, 2003) of moderately severe AD patients was evaluated. The results showed a statistically significant prevention of cognitive deterioration during a 36 week period in the more severely affected patients (baseline Alzheimer's Disease Assessment Scale – cognitive subscale ≥ 25). There was also a significant decline in plasma $\text{A}\beta_{42}$ in the CQ group compared with an increase in the placebo group. Subsequent clinical studies of this compound were not pursued due to manufacturing difficulties associated larger scale chemical synthesis.

A novel second generation MPAC PBT2 has been synthesized (Barnham *et al.*, 2003b) that has higher solubility and increased BBB permeability as compared with CQ. When tested in the APP/PS1 transgenic mouse model of AD (Adlard *et al.*, 2008) PBT2 decreased soluble interstitial $\text{A}\beta$ within hours, this was accompanied by improved cognitive performance. Moreover, there were significant decreases in insoluble $\text{A}\beta$ load and tau phosphorylation. A significant increase in synaptophysin levels was also observed indicating improved synaptic health. Following these successful animal studies PBT2 completed a randomized, double-blind, placebo-controlled Phase II clinical trial in 78 subjects with early AD over a 12 week treatment period. This study demonstrated safety and tolerability at 50 mg and 250 mg daily doses, and reduced CSF levels of $\text{A}\beta_{1-42}$ at the 250 mg dose. Improved cognitive performance in patients taking PBT2 (compared with placebo) was observed when tested on executive function in the Neuropsychological Test Battery (Lannfelt *et al.*, 2008; Faux *et al.*, 2010).

While PBT2 and CQ were originally identified as molecules capable of inhibiting $\text{A}\beta$ metal interactions recent results have suggested that these compounds are able to induce neuroprotective signalling cascades (White *et al.*, 2006). When 8-hydroxyquinolines coordinate metals the proton of the phenol group is lost such that a coordination complex of either Cu^{2+} or Zn^{2+} with two 8-hydroxyquinoline ligands results in a neutral hydrophobic complex (Figure 1) that is capable of crossing cell membranes (Caragounis *et al.*, 2007; Adlard *et al.*, 2008). Upon entering cells these complexes can induce metal-dependent activation of PI3K and JNK, resulting in JNK-mediated up-regulation of metalloprotease activity (White *et al.*, 2006; Caragounis *et al.*, 2007; Adlard *et al.*, 2008). These proteases are able to degrade $\text{A}\beta$. There is also an increase in phosphorylation of GSK3 β . This is an inhibitory phosphorylation of one of the kinases that phosphorylates the tau protein. The ability of the MPACs to inhibit toxic $\text{A}\beta$ metal interactions and also promote neuroprotective signalling by transporting these metals into cells

suggests that the actions of these compounds rather than acting as simple chelators is much more like that of metal chaperones where they protect against toxic reactions and at the same time promote beneficial interactions.

In addition to A β , Tau and GSK3 β have also been identified as potential therapeutic targets for AD. To date, the various strategies designed to target these proteins have been very much of a 'slice and dice' approach; that is, each protein has been targeted independently of the others. The data that are emerging from the MPAC strategy suggest that this is a more holistic approach to AD capable of having beneficial effects on a number of different protein targets implicated in the pathogenesis of AD.

The encouraging clinical results achieved by CQ and PBT2, has resulted in the metal hypothesis for AD gaining increased scientific support promoting a growing awareness of the therapeutic potential of targeting metal/A β -interactions. This in turn has prompted a number of groups to produce a diverse and growing array of metal chelating agents as potential therapeutic agents for AD. We will not attempt a comprehensive review of this literature here as this topic has recently been reviewed in depth (Smith *et al.*, 2007; Scott *et al.*, 2009; Perez *et al.*, 2010). Some of the more interesting compounds synthesized include high affinity metal chelators such as JKL169 (1,1'-xylyl bis-1,4,8,11 tetraaza cyclotetradecane) (Moret *et al.*, 2006) and chelators with additional functionality built into them. Examples of the additional functionality engineered into chelators include groups that promote blood-brain barrier uptake, for example, moieties such glucose have been added to 3-hydroxy-4-pyridone compounds (Schugar *et al.*, 2007). Other modifications include designing chelators to specifically target A β examples include XH1 ([[(4-benzothiazol-2-yl-phenylcarbamoyl)-methyl]-{2-[(2-[(4-benzothiazol-2-yl-phenylcarbamoyl)methyl]-carboxymethyl-amino)-ethyl]-carboxymethyl-amino)-ethyl]-amino)-acetic acid) (Dedeoglu *et al.*, 2004) and L2-b (N1,N1-dimethyl-N4-(pyridin-2-ylmethyl)benzene-1,4-diamine) (Choi *et al.*, 2010). Most of these compounds have been shown to be effective at inhibiting A β /metal interactions *in vitro* and some have also shown promise in transgenic animal studies. To date, there is no published clinical data on any of these chelators. Also, it is yet to be ascertained whether this array of compounds are capable of activating the neuroprotective pathways as do the MPACs and whether this will have significant implications for this class of therapeutic agent.

M(bis(thiosemicarbazone)) complexes

The mechanistic studies of the MPACs highlighted two potential modes of action, the chelating effects inhibiting A β toxicity and metal delivery inducing neuroprotective signalling. To ascertain whether the metal delivery aspects of the mechanism of action could have positive therapeutic benefits in the absence of directly targeting A β we identified the metal bis(thiosemicarbazone) (btsc) class of compounds as suitable candidates to evaluate this question (Figure 1). Btsc complexes have been investigated as metallodrugs for a number of years and proven to have a broad range of pharmacological activity (Beraldo *et al.*, 2004). In particular, recent interest

has focused on the use of btsc ligands as vehicles for the selective delivery of radioactive copper isotopes to hypoxic tissue and leukocytes in assessment of their potential as radiopharmaceuticals (Green *et al.*, 1988; Lewis *et al.*, 2001). Cu^{II}(btsc) complexes are stable (log $K_A \sim 18$) (Green *et al.*, 1988), neutral, low molecular weight compounds capable of crossing cell membranes. In some cases, it has been demonstrated that, once inside cells, Cu²⁺ is reduced by intracellular reductants to Cu⁺, which subsequently dissociates from the ligand (Kraker *et al.*, 1985; Adonai *et al.*, 2002). Other Cu^{II}(btsc) complexes are more resistant to reduction and dissociation and are trapped only in hypoxic cells. This selectivity is remarkably sensitive to the nature of alkyl groups attached to the diimine backbone of the ligand. For example, diacetyl-bis(N (4)-methyl-3-thiosemicarbazone) copper(II) (Cu^{II}(atmsm); Figure 1) features two methyl substituents on the backbone and copper is not released in normal cellular conditions. On the other hand, glyoxal-bis(N (4)-methyl-3-thiosemicarbazone) copper(II) (Cu^{II}(gtmsm); Figure 1) releases copper intracellularly (Fujibayashi *et al.*, 1997; Dearling *et al.*, 2002). The selective release of copper has been correlated with the Cu²⁺/Cu⁺ reduction potential because Cu^{II}(atmsm) is more difficult to reduce than Cu^{II}(gtmsm) (by some 160 mV) (Dearling *et al.*, 2002). However, differences in pK_a values, and the stability of the reduced state to dissociation of the metal may also be important.

A range of metal bis(thiosemicarbazone) complexes (M^{II}(btsc), where M = Cu^{II} or Zn^{II}) have been evaluated for their ability to increase intracellular metal levels in Chinese hamster ovary cells overexpressing APP (APP-CHO) and the subsequent effect on extracellular levels of A β (Donnelly *et al.*, 2008). The Cu^{II}(btsc) complexes were engineered to be either stable to both a change in oxidation state and dissociation of metal or susceptible to intracellular reduction and dissociation of metal. Treatment of APP-CHO cells with stable complexes resulted in elevated levels of intracellular copper with no effect on the detected levels of A β . Treatment with complexes susceptible to intracellular reduction increased intracellular copper levels but also resulted in a dose-dependent reduction in the levels of A β . Treatment with less stable Zn^{II}(btsc) complexes increased intracellular zinc levels with a subsequent dose-dependent depletion of A β levels. As with the MPACs the increased levels of intracellular bioavailable copper and zinc initiated a signalling cascade involving activation of PI3K and c-Jun N-terminal kinase and activation of proteases that degraded A β .

As both Cu^{II}(gtmsm) and Cu^{II}(atmsm) are capable of crossing the blood brain barrier (Fodero-Tavoletti *et al.*, 2010) the therapeutic potential of intracellular copper delivery was evaluated in the APP/PS1 transgenic AD model mice (Crouch *et al.*, 2009). Cu^{II}(gtmsm) which delivers bioavailable copper significantly inhibited GSK3 β in the brains of APP/PS1 transgenic AD model mice. Cu^{II}(gtmsm) also decreased the abundance of A β trimers and phosphorylated tau, and restored performance of AD mice in the Y-maze test to levels expected for cognitively normal animals. Improvement in the Y-maze correlated directly with decreased A β trimer levels. Cu^{II}(atmsm) does not deliver bioavailable copper and had no effects on cognition or disease relevant biomarkers. This study demonstrates that increasing intracellular copper bioavailability can restore cognitive function by inhibiting the accumulation of

neurotoxic A β trimers and phosphorylated tau (Crouch *et al.*, 2009).

PtLCl₂

It is generally accepted that the A β peptide is a valid target for therapeutic development and targeting the metal binding site of A β is an alternative to the metal chelating strategy of inhibiting A β /metal interactions that have been outlined above. Due to it being unstructured in the native state, the *de novo* design of effective inhibitors of A β is problematic. Therefore, a strategy of using metal compounds that target A β specifically by taking advantage of its intrinsic affinity for metal ions has been developed. The three histidine residues His-6, His-13 and His-14 are the principle A β metal binding ligands (Drew *et al.*, 2009a,b). Methylation of the imidazole side chains altered Cu binding and inhibited A β toxicity (Tickler *et al.*, 2005). Pt(II) complexes have been designed to target the A β -metal binding site. Pt(II) compounds are stable and essentially redox inert when present in biological systems. The slow kinetics associated with substitution reactions at the Pt(II) centre means that, once bound to a target, the Pt(II) metal is difficult to displace. The specificity of the interaction between Pt anticancer drugs and DNA has been attributed largely to the ability of the am(m)ine ligands to form hydrogen-bonds to guanine nucleotides of DNA. To promote specific binding to A β by Pt(II) complexes, a range of 1,10-phenanthroline ligands (L) were coordinated to Pt(II) to

help target the complex to N-terminal domain of A β . This was based on the observation that polyaromatic compounds bind to A β and inhibit its aggregation (Porat *et al.*, 2006). Moreover, the classic amyloid-binding fluorescent dyes Congo red and thioflavin T are also polyaromatic compounds. L-PtCl₂ complexes are highly stable and the likelihood of the chelating ligand, L, dissociating from the metal is remote. It has previously been demonstrated that the 1,10-phenanthroline ligands in the absence of metal bind weakly to A β via interactions with the aromatic residues Phe-4, Tyr-10 and Phe-19 on A β (Yao *et al.*, 2004). Importantly for this strategy, these residues span the metal binding residues His-6, His-13 and His-14.

A range of L-PtCl₂ complexes were examined in a variety of *in vitro* assays and shown to bind to A β , inhibit neurotoxicity and rescue A β -induced synaptotoxicity in mouse hippocampal slices (Barnham *et al.*, 2008). Coordination of the complexes to A β altered the chemical properties of the peptide inhibiting amyloid formation and the generation of ROS. In comparison, the classic anticancer drug cisplatin did not affect any of the biochemical and cellular effects of A β . This implies that the planar aromatic 1,10-phenanthroline ligands L confer some specificity for A β onto the platinum complexes. The potent effect of the L-PtCl₂ complexes identifies this class of compounds as potential therapeutic agents for AD. However, the major challenge for the future development of this class of compound is designing compounds that are able to cross the blood brain barrier in sufficient quantity to be an effective agent *in vivo*. Subsequent to this study it has

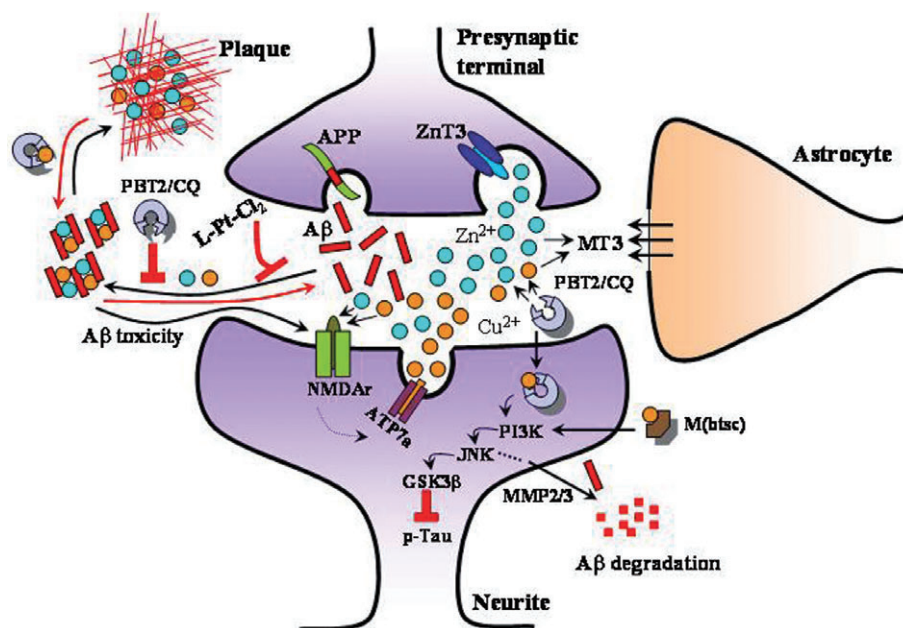


Figure 2

A schematic representation of the synapse and the role that 'free' Cu and Zn play in synaptic transmission via modulating N-methyl-D-aspartate receptor (NMDAr) function. When amyloid- β (A β) interacts with these metals the peptide aggregates forming toxic oligomers and ultimately amyloid plaques. The toxicity of oligomers is elicited through interactions with the glutamatergic receptors such as the NMDAr. This system offers a number of potential therapeutic strategies. These include the MPACs such as clioquinol (CQ) and PBT2 which inhibit A β /metal interactions and facilitate neuroprotective signalling. M(bis(thiosemicarbazone)) (M(bisc)) complexes can also induce neuroprotective signalling. The L-PtCl₂ complexes are able to bind to A β to inhibit the formation of toxic species.

been demonstrated that ruthenium (Ru) complexes (Valensin *et al.*, 2010) with aromatic ligands were also able to have anti-A β effects *in vitro*.

Concluding remarks

Although more than 100 years have passed since the first reports of AD, development of effective treatments remains challenging as AD pathogenesis is a complex process involving many factors. Metal ions play essential role in a number of essential neurological processes and therefore a breakdown in the homeostatic systems that regulate them can have catastrophic consequences (Figure 2). When A β peptide interacts with metal ion not only is there the potential to cause such a breakdown in metal homeostasis but also the generation of toxic A β aggregates. Interdicting in these processes has shown great potential in both animal models of AD and in early stage clinical trials.

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Conflict of interest

K. J. B. is a consultant to and share holder of Prana Biotechnology LTD. The company owns and is developing PBT2 as a therapy for AD.

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