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Therapeutic Redistribution of Metal Ions To Treat Alzheimer's Disease

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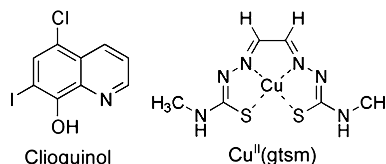
CONSPECTUS

Currently, therapeutics that modify Alzheimer's disease (AD)are not available. Increasing age is the primary risk factor for AD and due to an aging global population the urgent need for effective therapeutics increases every year. This Account presents the development of an AD treatment strategy that incorporates diverse compounds with a common characteristic: the ability to redistribute metal ions within the brain.



Central to cognitive decline in AD is the amyloid-***β*** peptide (A***β***) that accumulates in the AD brain. A range of therapeutic strategies have been developed based on the premise that decreasing the brain A***β*** burden will attenuate the severity of the disease symptoms. Unfortunately these treatments have failed to show any positive outcomes in large-scale clinical trials, raising many questions regarding whether therapeutics for AD can rely solely on decreasing A***β*** levels.

An alternate strategy is to target the interaction between A***β*** and metal ions using compounds with the potential to redistribute metal ions within the brain. The original rationale for this strategy came from studies showing that metal ions promote A***β*** toxicity and aggregation. In initial studies using the prototype metal-chelating compound clioquinol (CQ), CQ prevented A***β*** toxicity *in vitro*, out-competed Aβ for metal ions without affecting the activity of metal-dependent enzymes, and attenuated the rate of cognitive decline in AD subjects in a small phase II clinical trial. All these outcomes were consistent with the original hypothesized mechanism of action for CQ where prevention or reversal of the extracellular Aβ−metal interactions could prevent Aβ toxicity.

Soon after the completion of these studies, a new body of work began to suggest that this hypothesized mechanism of action for CQ was simplistic and that other factors were also important for the positive therapeutic outcomes. Perhaps most significantly, it was shown that after CQ sequesters metal ions the neutral CQ−metal complex crosses cell membranes to increase intracellular levels of the metals, thereby initiating protective cell signaling cascades. The activity of CQ therefore appeared to be two-fold: it prevented toxic interactions between A***β*** and metal ions outside the cell, and it redistributed the metal ions into the cell to promote healthy cell function.

To determine the significance of redistributing metal ions into the cell, glyoxalbis(*N*(4)-methylthiosemicarbazonato)Cu^{II} [Cu^{II}(gtsm)] was tested in models of AD. Cu^{II}(gtsm) delivers Cu into cells, but, unlike CQ, it cannot out-compete Aβ for metal ions. When tested in AD model mice, the Cu^{II}(gtsm) treatment restored cognitive function back to levels expected for cognitively healthy mice. The most advanced compound from this therapeutic strategy, PBT2, can sequester metal ions from Aβ and redistribute them into the cell like CQ. PBT2 improved cognition in a phase II clinical trial with AD patients, and further clinical testing is currently underway.

Introduction

Alzheimer's disease (AD) is a fatal neurodegenerative disorder that predominantly affects the elderly. The symptoms of memory loss and behavioral abnormalities escalate in severity as the disease progresses over many years, and the people affected need ongoing care. An aging global population means that the social and economic burden of AD will increase rapidly in the coming years. Although urgently needed, the development of disease-modifying therapeutics to treat AD has eluded researchers for decades.

Central to the cognitive deficits of AD is the amyloidogenic amyloid-β peptide (Aβ) that accumulates in the AD affected brain.[1] Post-mortem identification of extracellular amyloid deposits in the brain remains the only definitive way to diagnose AD, and in the 1980s Aβ was identified as the primary protein component of these deposits.[2−4] Following on from this, the toxic activity of Aβ rapidly became one of the most widely studied areas of neuroscience research. Most of the potential therapeutic strategies to treat AD were developed based on the premise that

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decreasing Aβ burden in the brain should attenuate disease symptoms. Unfortunately most of these strategies have ultimately failed to show disease-modifying outcomes in large scale trials (see ref 5 for review), causing many to question the validity of Aβ as a therapeutic target. The therapeutics tested to date include inhibitors of Aβ production, anti-Aβ immunotherapy, and therapeutics that promote Aβ clearance. Consistent in all these approaches is that their proposed mechanism of action was restricted to a direct effect on Aβ levels in the brain, and the negative results have lead some to suggest that therapeutics for AD cannot rely on decreasing Aβ levels in isolation. However it should also be noted that most of the treatments that failed were poor drugs that lacked the ability to cross the blood–brain barrier, were toxic, or had dubious mechanisms of action. That these compounds were pursued into large and expensive late stage clinical trials probably raises more questions about Pharma's decision making processes than it does about the Aβ centric hypothesis of AD.

Despite these misgivings, it is becoming clear that merely inhibiting Aβ is not sufficient to reverse the degeneration in the brain that occurs as the disease progresses. This has resulted in a growing momentum for clinical trials of anti-Aβ therapies to be staged in subjects at the earliest stages of the disease before significant degeneration has occurred. In many cases it is being advocated that trials will need to be carried out at what we currently recognize as a presymptomatic stage of the disease. However there are two problems with this strategy. The first is that we currently lack reliable biomarkers to allow us to accurately identify presymptomatic target individuals. One approach to circumventing this is to conduct trials involving subjects whose families have rare genetic forms of AD, where the natural course of the disease is more predictable and Aβ deposition in the brain within a relatively short time frame is certain. The caveat with this approach is that while it may identify therapeutics effective in treating familial forms of the disease, whether this would translate to the predominant sporadic form of the disease is uncertain. The second problem of this strategy is that it offers little or no hope to those who are actually diagnosed with AD. Therefore we would advocate that effective therapeutics for AD need to be more integrated, targeting not just Aβ but other pathological features of the disease as well as holding out some hope of regenerating the lost tissue. This Account relates our attempts to generate such a therapy.

TABLE 1. Proposed Mechanisms by Which Aβ Interaction with the Metal Ions Zinc (Zn) and Copper (Cu) Promotes the Neurotoxic Activity of Aβ

proposed mechanism of metal-mediated Aβ toxicity	ref
Inhibition of mitochondrial function	9
Generation of H_2O_2	11
Disruption of lipid membranes	10
Formation of neurotoxic Aβ aggregates	12
Formation of synaptotoxic Aβ oligomers	13

Preventing Aβ–Metal Interactions: A New Therapeutic Strategy

During the 1990s, a number of publications, primarily from Ashley Bush and co-workers showed that Aβ reacts with transition metal ions, Zn, Cu, and Fe, to recapitulate many of the pathological features observed in AD. This was primarily peptide aggregation and the generation of reactive oxygen species that would contribute to the oxidative stress observed in the AD brain.[6−8] By incubation of synthetic Aβ with metal ions, it was shown zinc (Zn) and copper (Cu) ions could induce rapid precipitation of the Aβ,[7] thus providing an important insight to the biochemical conditions that may promote Aβ aggregation in the AD brain. A number of subsequent *in vitro* studies then generated evidence to indicate the mechanisms by which Aβ–metal interactions may contribute to neuronal dysfunction in AD[9−13] (Table 1), and *in vivo* data demonstrated that Aβ aggregation in the brain is dependent on the availability synaptic Zn.[14] Further to this, analyses of the AD-affected brain have revealed that Cu, Zn, and Fe accumulate in extracellular amyloid plaques.[15] Collectively, these data supported the rationale for developing metal-chelating compounds as a new therapeutic strategy for treating AD; if interactions between Aβ and metal ions contributed to the toxicity and deposition of Aβ in the AD brain, therapeutics designed to prevent this interaction should be protective.

Numerous *in vitro* studies showing that cell-impermeable chelators such as ethylenediaminetetraacetic acid could prevent metal ion induced Aβ aggregation[6−8] provided the initial support for the strategy of preventing extracellular Aβ–metal interaction. The first *in vivo* support came in 2001 from a study that administered 5-chloro-7-iodo-8-hydroxyquinoline (clioquinol, CQ, Figure 1) to the Tg2576 mouse model of AD.[16] Tg2576 mice are a widely used mouse model of AD because transgenic expression of a human form of the Aβ precursor protein causes Aβ to accumulate in the brain, causing a progressive memory impairment.[17] CQ was chosen for the initial *in vivo* study because of its selectivity as a chelator of Zn^{2+} and Cu^{2+} compared with

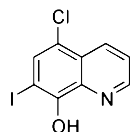

FIGURE 1. Structure of 5-chloro-7-iodo-8-hydroxyquinoline (clioquinol/CQ).

Ca^{2+} and Mg^{2+}[16] and because of its ability to cross the blood–brain barrier.[18] Analyses of brains from CQ treated Tg2576 mice revealed fewer amyloid plaques and a 41% decrease in insoluble Aβ.[16] Together with parallel *in vitro* data this indicated CQ was effective in preventing the accumulation of insoluble, aggregated Aβ in the brain by preventing the ability for Aβ to interact with metal ions.

At the time of these studies, the relative contribution of soluble and insoluble Aβ to neuronal failure was not yet fully resolved, but the idea that soluble Aβ was more toxic than insoluble Aβ had emerged.[19,20] An immediate concern therefore was that liberating Aβ from an inert, nontoxic, pool of insoluble Aβ by stripping away the metal ions that induced its precipitation would increase the amount of toxic, soluble Aβ in the brain. The concentration of soluble Aβ was increased in the brains of the CQ treated Tg2576 mice, but quantitatively these increases were minor; the insoluble pool of Aβ in the CQ treated mice decreased by 375.4 μg/g brain wet weight compared with the soluble pool, which increased by 2.5 μg/g wet weight.[16] These results implied that after metal ions had been removed from the insoluble Aβ the solubilized Aβ was then rapidly cleared from the brain. It took several years before mechanistic studies supporting this possibility were published. In an *in vitro* study using synthetic Aβ, it was shown that Aβ in the absence of Zn is readily degraded by a range of proteases normally present in the brain, but when the Aβ was able to interact with Zn, the Aβ became highly resistant to protease degradation due to Zn-induced conformational changes.[21] These conformational changes were reversed by adding CQ, thus restoring Aβ sensitivity to protease-mediated degradation.[21] It was therefore concluded from the *in vitro* study that the absence of an increase in soluble forms of Aβ in the brains of the CQ treated Tg2576 mice[16] was because metal-free soluble Aβ is highly sensitive to the activity of proteases that already exist within the brain.[21]

Following the promising outcomes for CQ in the Tg2576 mouse study,[16] a pilot phase II clinical trial with AD subjects was undertaken. While the primary objective of this study was to determine safety and tolerability of the treatment, clinical data collected from the small number of subjects showed that the rate of cognitive deterioration was significantly attenuated by the CQ treatment.[22]

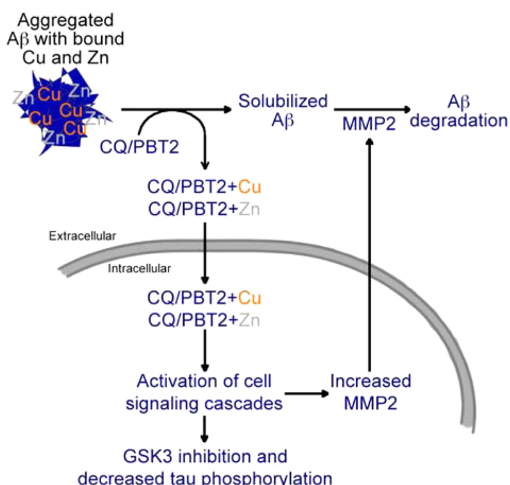

FIGURE 2. Dual mechanism of action for CQ and PBT2. Consistent with the original rationale for testing CQ and PBT2 as potential AD therapeutic agents, CQ and PBT2 are able to prevent the formation of metal-induced Aβ aggregates and solubilize metal-induced Aβ oligomers by sequestering the metals away from the Aβ. In addition to these extracellular activities, CQ and PBT2 are able to translocate metal ions into the cell. The consequence of this metal redistribution is the activation of cell signaling pathways. Abbreviations: Aβ, amyloid-β; CQ, clioquinol; MMP2, matrix metalloprotease 2.

A New Perspective on the Mechanism of Action

Prior to 2004, the preclinical and clinical studies with CQ all appeared to support a relatively simple therapeutic mechanism of action: attenuate Aβ toxicity, and therefore the symptoms of AD, by preventing or reversing extracellular interaction between Aβ and metal ions. However our attempts to develop new compounds based on this proposed mechanism of action were proving unreliable, suggesting that the concept of CQ working solely by inhibiting Aβ/metal interactions was simplistic. This started to become more apparent when two related studies presented data to indicate that CQ was able to induce other biological phenomena.[23,24] The first study[23] examined the effects of CQ on the levels of Cu in yeast cells. Adding CQ to the yeast cell culture medium promoted cellular uptake of Cu.[23] This study did not examine the effects of the CQ on Aβ, but it did propose for the first time that, in addition to preventing extracellular Aβ–metal interactions, the ability for CQ to redistribute Cu into cells may contribute to the compound's activity as an AD therapeutic. Continuing on this theme, the second study[24] examined the cellular effects of CQ-mediated translocation of metal ions into cells.

Preformed CQ−Cu and CQ−Zn complexes were exposed to mammalian cells grown in culture along with the control treatments of CQ on its own or the metal ions on their own. By measuring metal content of the cells after a short incubation period the researchers found, as per the yeast study,[23] that CQ promoted cellular uptake of the metal ions.[24] The cellular response to this was an up-regulated cell signaling cascade involving a number of molecules with known neuroprotective activity[24] (Figure 2). One downstream effect of this signaling cascade was the up-regulation of cellular proteases that degraded Aβ,[24] thus demonstrating that the metal chaperone activity of CQ promoted the cell's own capacity to degrade Aβ. Perhaps more importantly, one of the kinases included in the CQ−metal-induced cell signaling cascade was glycogen synthase kinase-3 (GSK3). GSK3 is a ubiquitous kinase with a large range of diverse substrates, including the microtubule-associated protein tau.[25,26] Tau is hyperphosphorylated in the AD brain, and many reports indicate excessive GSK3 activity contributes to this hyperphosphorylation (reviewed in ref 27). The *in vitro* study examining the effects of CQ−metal complexes on cells[24] therefore demonstrated that by facilitating cellular uptake of Cu and Zn CQ was able to promote Aβ degradation and potentially decrease tau phosphorylation, thereby hitting the two primary pathological hallmarks of the AD brain.

Increasing Intracellular Levels of Bioavailable Cu

The technique used to measure CQ-facilitated cellular uptake of Cu and Zn in the initial *in vitro* studies was inductively coupled mass spectrometry (ICP-MS).[23,24] This technique allows for sensitive measurement of multiple metal ions within biological samples, but it does not differentiate between the multitude of pools within which these metal ions can exist in the cell. Therefore, while CQ was shown to facilitate cellular uptake of the metal ions, the ICP-MS data generated could not be used to identify whether the metal ions were released from the CQ inside the cell to increase their bioavailability or whether the CQ−metal complexes remained intact within the cell. The binding affinities of CQ for Cu and Zn ($K_d \approx 10^{-10}$, 10^{-8}, respectively) suggest that the metal complexes are unlikely to prevent the metal ions being coordinated by various metal chaperones within the cell such as metallothionein.

To shed some light on the question of whether the activity of CQ−metal complexes involved intracellular dissociation of the complex to increase levels of bioavailable metal, studies using metal complexes of bis(thiosemicarbazone) ligands were

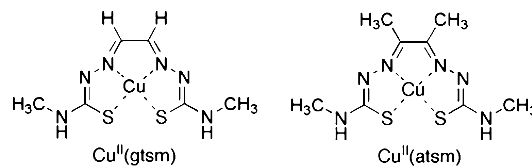

FIGURE 3. Structures of glyoxalbis(*N*(4)-methylthiosemicarbazonato)−Cu^{II} [Cu^{II}(gtsm)] and diacetylbis(*N*(4)-methyl-3-thiosemicarbazonato)−Cu^{II} [Cu^{II}(atsm)].

undertaken. The bis(thiosemicarbazone) ligands coordinate metal ions such as Cu and Zn via an N_2S_2 ligand donor set and have been studied as metallodrugs for a number of years.[28−31] The bis(thiosemicarbazonato)−Cu^{II} complexes are stable (K_d[extracellular] $\approx 10^{-18}$),[32] neutral, low molecular weight compounds capable of crossing the blood−brain barrier.[29,33,34] Furthermore, the bis(thiosemicarbazone) ligands are highly amenable to chemical modification allowing for subtle control of intracellular metal release/retention properties. Illustrating this point are the two closely related compounds glyoxalbis(*N*(4)-methylthiosemicarbazonato)−Cu^{II} [Cu^{II}(gtsm)] and diacetylbis(*N*(4)-methyl-3-thiosemicarbazonato)−Cu^{II} [Cu^{II}(atsm)] (Figure 3). The electron-donating methyl groups on the atsm ligand lower the Cu^{II}/Cu^{I} reduction potential for Cu^{II}(atsm) when compared with Cu^{II}(gtsm) (E_m = 0.60 mV and E_m = 0.44 mV, respectively versus SCE where Fc/Fc+ = 0.54 V).[32,35] So while the Cu^{II} on Cu^{II}(gtsm) is readily reduced in the presence of intracellular reductants causing dissociation of the complex inside the cell and the release of the lower affinity Cu^{I} (K_d[intracellular] $\approx 10^{-8}$)[32] making the metal bioavailable, the Cu^{II} on Cu^{II}(atsm) is more resistant to intracellular reduction and the Cu^{II}(atsm) is therefore less likely to dissociate under normal cellular conditions. This feature was exploited in the first study designed to identify whether the therapeutic activity of compounds that delivered metal ions into cells involved intracellular dissociation of the metal−ligand complex.[36] Cells grown in culture were treated with either Cu^{II}(gtsm) or Cu^{II}(atsm), then the cells were measured for Cu content and biochemical markers of activated cell signaling pathways. ICP-MS analyses revealed both compounds increased cellular Cu content approximately 200-fold demonstrating both compounds readily delivered Cu into the cell comparable to CQ.[36] However, only the Cu^{II}(gtsm) treatment, the treatment expected to increase levels of bioavailable Cu in the cell under normal cellular conditions, was found to elicit cellular signaling cascades similar to those previously observed for CQ−metal complexes.[36]

Cu^{II}(gtsm) and Cu^{II}(atsm) were subsequently tested in the APP/PS1 transgenic mouse model of AD.[37] The rationale for

this study was to use Cu^{II}(gtsm) to determine whether the therapeutic activity of compounds like CQ in AD model mice was limited to their ability to prevent extracellular Aβ–metal interactions or whether the induced signaling cascades also contribute to *in vivo* neuroprotection. At the time, a number of other compounds capable of sequestering metal ions away from Aβ (PBT2 (see below), pyrrolidine dithiocarbamate (PDTC), and DP-109) had already been shown to decrease the abundance of amyloid plaques in the brains of AD model mice,[38–40] and of these, PBT2 and PDTC had both been shown to improve cognitive function in AD model mice.[38,39] Although treatment with PDTC had been shown to induce the same cell signaling responses as CQ, it remained unclear whether the improved cognitive function in PDTC (and PBT2) treated mice involved a metal chaperone mechanism of action in which metal ions were redistributed away from the extracellular Aβ and into the cells. APP/PS1 mice were therefore treated with Cu^{II}(gtsm) or Cu^{II}(atsm) then tested for cognitive function. Compared with the Cu^{II}(atsm) and placebo treatments, Cu^{II}(gtsm) increased cognitive performance in the AD model mice to levels expected for cognitively healthy mice.[37] Parallel analyses of brain material dissected from the mice and cells grown in culture provided data consistent with the Cu^{II}(gtsm) mechanism of action involving intracellular release of the Cu from the gtsm ligand. These data included the activation of cell signaling pathways that led to the inhibition of GSK3 and a decrease in tau phosphorylation.[37] It was also demonstrated that the Cu^{II}(gtsm) treatment decreased levels of Aβ oligomers in the brains of the APP/PS1 mice and that the decreased oligomer levels correlated directly with improved cognitive function of the mice.[37] Furthermore, the Cu^{II}(gtsm) treatment did not alter the abundance of extracellular amyloid plaques.[37] This study therefore provided the first data to indicate cognitive deficits in AD model mice could be treated using a compound that increases intracellular levels of bioavailable Cu in the brain without necessarily directly attenuating extracellular Aβ–metal interactions. By showing that the Cu^{II}(gtsm) treatment inhibited GSK3, decreased Aβ oligomer levels, and decreased tau phosphorylation, this study also demonstrated that increasing levels of bioavailable metal ions within the cell represents an integrated approach to attenuating cognitive deficits.

From Clioquinol to PBT2

Despite the positive outcomes for CQ in its clinical trial as a therapeutic for AD,[22] the further development of this compound was not progressed due to manufacturing difficulties. A small contamination of di-iodo-8-hydroxyquinoline, a known carcinogen, occurred during the larger scale chemical synthesis. Because the second generation PBT2 (a novel chemical entity) was then ready for clinical development, no attempt was made to pursue further work with CQ.

PBT2 is a novel 8-hydroxyquinoline derivative (K. Barnham, E. Gautier, G. Kok, and G. Krippner 2003, 8-Hydroxy Quinoline Derivatives [World, Prana Biotechnology Ltd.], PCT No. PCT/AU03/00914, Publication No. WO2004007461, patent pending). In addition to the large scale synthesis difficulties associated with CQ described above, PBT2 was adopted for further clinical development instead of CQ because of its better solubility, increased ability to cross the blood–brain barrier, and improved efficacy in preclinical *in vivo* studies.[38] Importantly PBT2 is more efficient in delivering metal ions across biological membranes. As an 8-hydroxyquinoline, PBT2 coordinates Cu and Zn in a 2:1 ratio, and this is accompanied by a deprotonation of the phenol proton. This results in PBT2 forming a neutral, soluble complex with metal ions that is capable of crossing cellular membranes. A phase IIa clinical trial of PBT2 has shown that treatment for 12 weeks improved measures of cognitive function in patients with mild AD.[41–43] Like CQ, *in vitro* and *in vivo* studies indicate that the therapeutic efficacy of PBT2 is based, at least in part, on the compound's ability to deliver bioavailable Cu and Zn into cells.[38,44,45] In addition to the activation of cell signaling pathways with neuroprotective potential,[45] the consequences of this metal delivery mechanism of action have also been shown to include the promotion of neurite extension and increased dendritic spine density.[44]

Consistent with the original rationale for developing AD therapeutics that inhibit or reverse the interaction between Aβ and metal ions, PBT2 is able to inhibit Zn-induced formation of protease-resistant Aβ aggregates. In the absence of Zn, Aβ is rapidly degraded by matrix metalloprotease 2 (MMP2), but in the presence of Zn, the Aβ forms protease-resistant aggregates.[45] The formation of these protease-resistant aggregates is prevented by PBT2.[45] These data, together with similar data from experiments using CQ,[21] demonstrate an important feature of PBT2 and CQ as potential AD therapeutics; the affinity of the compounds for Cu and Zn is high enough to inhibit Aβ/metal interactions but not so high that it inhibits the actions of essential metal-dependent enzymes. The MMP2 enzyme that degrades Aβ requires Zn for optimal hydrolytic function.[46] The data presented for PBT2 and CQ[21,45] therefore demonstrate that while the metal binding activity of PBT2 and CQ is strong

enough to inhibit Aβ/Zn interactions it does not impair the normal Zn-dependent activity of MMP2.

Increasing Bioavailable Cu or Zn?

When CQ or PBT2 are coordinated to either Cu or Zn prior to treating cells, the signaling changes elicited by the ligand–Cu complexes are the same as those elicited by the ligand–Zn complexes.[24,45,47] Similarly, Zn complexes of bis(thiosemicarbazone) ligands induce the same changes as Cu complexes of bis(thiosemicarbazone) ligands when exposed to cells grown in culture.[36] A recent study has shed light on one possible cellular mechanism that could explain this convergence of activities by bioavailable Cu and Zn. This study examined the cellular conditions that contribute to the reduction and intracellular release of Cu from bis(thiosemicarbazone) ligands using cells transfected with a metal-responsive element (MRE)–luciferase construct to detect increases in intracellular, bioavailable Cu.[48] An increase in bioavailable metal within the cell triggers the MRE component of the promoter that drives expression of the transgenic luciferase gene (detectable by increased luminescence of the cell lysates), and this construct had been used in previous studies to monitor intracellular increases in bioavailable Cu.[49,50] By use of this construct, it was shown that after treatment of cells with Cu^{II}(gtsm) or Cu^{II}(atsm) under normal conditions, only the Cu^{II}(gtsm) induced an increase in luminescence in the cell lysates,[48] a result consistent with Cu^{II}(atsm) being resistant to reduction and intracellular dissociation under normal conditions. Central to the activity of the MRE–luciferase construct is the metal-responsive transcription factor-1 (MTF-1).[50] MTF-1 is up regulated by an increase in bioavailable Zn within the cell, and the MTF-1 in turn binds to and activates the MRE.[50] So while the MRE–luciferase construct has been used to monitor increases in bioavailable Cu, the mechanism by which the construct works requires Cu-mediated displacement of Zn from endogenous pools, most likely metallothioneins.[50] Increased luminescence detected in cells treated with Cu^{II}(gtsm)[48] was therefore a measure of the treatment indirectly increasing bioavailable Zn in the cell. Based on this, the commonality between complexes capable of delivering bioavailable Cu and Zn, in terms of the cellular responses to the treatments, may reside in whether or not the compounds directly or indirectly increase bioavailable Zn within the cell (Figure 4). A likely mechanistic consequence of this is Zn-mediated inhibition of phosphatase activity. A broad range of phosphatases are sensitive to Zn, including calcineurin, which has been implicated in AD pathology, and

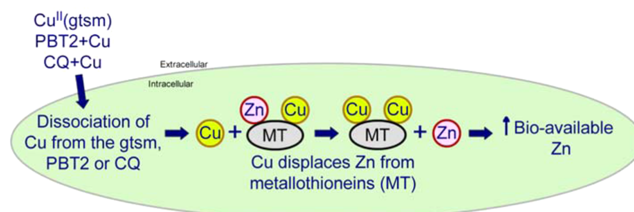

FIGURE 4. Potential mechanism by which delivering bioavailable Cu into the cell using CQ, PBT2, or Cu^{II}(gtsm) increases levels of bioavailable Zn within the cell. Unlike Cu^{II}(gtsm), CQ and PBT2 are also able to deliver Zn directly into the cell. Abbreviations: MT, metallothioneins; CQ, clioquinol.

inhibition of calcineurin has been implicated in the ability for CQ, PBT2, and bis(thiosemicarbazone) ligands to activate neuroprotective signaling cascades.[45,51,52] Whether or not the initial increase in bioavailable Cu within the cell has the direct ability to promote neurotrophic cellular responses, independently of its effects on cellular levels of bioavailable Zn, remains to be determined.

The promising results achieved by CQ and PBT2 has led to a growing awareness of the therapeutic potential of targeting Aβ/metal 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. Space constraints do not allow us to attempt a comprehensive review of this literature here, but this topic has recently been reviewed in depth.[53,54] In general these compounds have been shown to be effective at inhibiting Aβ/metal interactions *in vitro*, and some have also shown positive results in transgenic animal studies. In most cases, it is yet to be determined whether these compounds can act as metal chaperones like CQ and PBT2 to activate the neuroprotective pathways and whether this will have any implications for the clinical efficacy of this class of therapeutic agent.

Summary

Cognitive function is based on the transfer of chemical signals from one neuron to the next via the process of synaptic transmission. Synaptic transmission involves the coordinated function of neurotransmitters, cell surface receptors, and pre- and postsynaptic cycling of synaptic vesicles. The role of metal ions in this dynamic process is becoming increasingly clear (reviewed in ref 55), and the aberrant sequestration of these essential metal ions into extracellular pools of Aβ has been proposed as an important factor contributing to synaptic dysfunction in the AD brain.[56] Indeed, the formation of synaptotoxic Aβ oligomers is driven

by Zn released into the synapse, a process that can be prevented using CQ.[13]

To date, attempts at dissecting out specific aspects of AD biology and targeting them in isolation have failed as therapeutic strategies. The use of compounds with the ability to remove metal ions from Aβ and redistribute them within the brain to regions or cellular locations where they can promote synaptic function offers the opportunity to take a more integrated approach to treating AD. These molecules not only promote Aβ degradation but also inhibit GSK3, tau phosphorylation, and calcineurin activity. Furthermore, their ability to increase levels of bioavailable Cu and Zn within the cell is likely to replenish the pools of these metal ions needed for synaptic function. Targeting multiple aspects of AD biology holds greater therapeutic potential than methods designed to treat just individual components of this complex disease.

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ABBREVIATIONS

AD, Alzheimer's disease; Aβ, amyloid-β peptide; CQ, clioquinol; Cu, copper; Cu^{II}(atsm), diacetylbis(4-methylthiosemicarbazonato)Cu^{II}; Cu^{II}(gtsm), glyoxalbis(*N*(4)-methylthiosemicarbazonato)Cu^{II}; GSK3, glycogen synthase kinase 3; MMP2, matrix metalloprotease 2; Zn, zinc;

BIOGRAPHICAL INFORMATION

Peter Crouch received his B.Sc. (Hons) in 1997 and his Ph.D. in plant physiology in 2002 from La Trobe University. After completing his Ph.D., he switched his research focus to neurodegenerative diseases and took up a research position in 2003 at the Centre for Neuroscience. He joined the Department of Pathology, University of Melbourne, in 2006 where he currently leads a research group that undertakes preclinical *in vivo* and *in vitro* testing of therapeutics for neurodegenerative diseases.

Kevin Barnham received his B.Sc. in Chemistry from the University of Queensland in 1986 and his Ph.D. in 1993. His doctoral worked focused on NMR spectroscopy to study the interactions of Pt anticancer drugs with amino acids and nucleobases under the supervision of Trevor Appleton. His postdoctoral work (1992−1995) under the supervision of Peter Sadler at The University of London expanded on this work. In 1995, he joined the Biomolecular Research Institute, The University of Melbourne, where he used NMR spectroscopy to determine the structures of proteins as potential drug targets. Since 2001, his research has focused on developing new therapeutic strategies for neurodegenerative diseases.

FOOTNOTES

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The authors declare the following competing financial interest(s): K. Barnham is a share holder and consultant to Prana Biotechnology.

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