Chapter 12

# Amyloid-β Metal Interaction and Metal Chelation

Math P. Cuajungco, [1]Christopher, J. Frederickson and [2]Ashley I. Bush
*Harvard Medical School and Massachusetts Eye and Ear Infirmary, Boston MA 02114 USA..*
*[1]NeuroBioTex, Inc. and University of Texas Medical Branch, Galveston TX 77550 USA.*
*[2]Harvard Medical School and Massachusetts General Hospital, Boston MA 02114 USA*

Abstract: Alzheimer's disease (AD) is associated with the abnormal aggregation of amyloid-beta (Aβ) protein. Aβ and its precursor protein (APP) interact with metal ions such as zinc, copper and iron. Evidence shows that these metals play a role in the precipitation and cytotoxicity of Aβ. Despite recent advances in AD research, there is a lack of therapeutic agents to hinder the apparent aggregation and toxicity of Aβ. Recent studies show that drugs with metal chelating properties could produce a significant reversal of amyloid-β plaque deposition *in vitro* and *in vivo*. Here we discuss the interaction of Aβ with metals, metal dyshomeostasis in the CNS of patients with AD, and the potential therapeutic effects of metal chelators.

Key words: Metal chelators, Zinc, Copper, Iron

## 1. INTRODUCTION

Alzheimer's disease (AD) is characterized by progressive loss of cholinergic neurons with concomitant deterioration of memory and cognition (Arendt *et al.*, 1984; Winblad *et al.*, 1985; Hyman *et al.*, 1986; Hu *et al.*, 2003; Luth *et al.*, 2003). In AD brain, there is marked accumulation of amyloid-β (Aβ) protein, the main constituent of senile plaques, particularly its longer and more amyloidogenic form Aβ1-42 (Glenner and Wong, 1984; Citron *et al.*, 1997). Evidence shows that formation of neurofibrillary tangles (NFT) and neuropil threads occur subsequent to amyloid

deposition (Lombardo *et al.*, 2003). The Aβ protein is generated from the amyloid precursor protein (APP) by the proteolytic activities of β- and γ-secretases (Wolfe, 2003; Yang *et al.*, 2003). Majority of AD cases are classified as sporadic and approximately 5% of AD patients suffer from an early-onset familial AD (FAD) form. Mutations of the APP (chromosome 21), and presenilin (chromosomes 1 and 14) genes are linked to forms of FAD (Levy-Lahad *et al.*, 1995; Tanzi *et al.*, 1996; Levy-Lahad *et al.*, 1998). Several risk factors for late-onset AD have been identified such as apolipoprotein E (apo-E; ε4 allele) on chromosome 19 (Roses *et al.*, 1995) and $\alpha_2$-macroglobulin (A2M) gene on chromosome 12 (Blacker *et al.*, 1998; Saunders *et al.*, 2003). In addition, a genetic locus on chromosome 10 was identified that may be also a risk factor for late-onset AD (Bertram *et al.*, 2000; Ertekin-Taner *et al.*, 2000).

The pathophysiologic effects of FAD gene mutations result in increased production of Aβ protein and its subsequent cerebral deposition, especially of Aβ1-42 isoform (Citron *et al.*, 1992; Citron *et al.*, 1997). Note, however, much is not known how amyloid plaques can form and accumulate in AD brain without any genetically determined elevation in Aβ synthesis. Furthermore, overproduction of Aβ1-42 associated with FAD or Down's syndrome (Citron *et al.*, 1992; Lemere *et al.*, 1996; Citron *et al.*, 1997) does not fully explain its pathologic deposition in the brain, suggesting that other neurochemical factors initiate Aβ deposition for FAD and sporadic or late-onset AD. Mitigating factors that may initiate amyloid deposition are the pathologic interactions of cerebral Aβ with transition metals such as zinc, copper or iron (Bush *et al.*, 1994b; Huang *et al.*, 1997; Atwood *et al.*, 1998). Zinc, copper, and iron have been implicated as possible pathogenic agents in AD due to high concentration gradients of these metals in the cortex, hippocampus and the cortical vasculature (Smith, 1983; Dwork *et al.*, 1988; Frederickson, 1989); brain regions that are severely affected by the pathological lesions of AD (Goedert *et al.*, 1991). Studies performed in our laboratory, as well as by other groups clearly demonstrated that these metals interact and induce Aβ protein precipitation (Lovell *et al.*, 1998; Garzon-Rodriguez *et al.*, 1999; Atwood *et al.*, 2000a; Cuajungco *et al.*, 2000b; Hirakura *et al.*, 2000; Miura *et al.*, 2000; Yang *et al.*, 2000; Kozin *et al.*, 2001; Suzuki *et al.*, 2001; Yoshiike *et al.*, 2001). The role of metals in AD pathogenesis remains unclear despite numerous reports and hypotheses that attempt to link them with AD. Notwithstanding, there is compelling evidence that chelation of transition metal, which prevents their promiscuous interactions with Aβ protein, has therapeutic potential for AD pathology.

## 2. CEREBRAL METAL ACCUMULATION IN ALZHEIMER'S DISEASE

Previous studies that attempt to quantify cerebral, cerebrospinal fluid (CSF), or peripheral levels of zinc, copper and iron in AD produce highly variable results (Hershey *et al.*, 1983; Sahu *et al.*, 1988; Basun *et al.*, 1991; Constantinidis, 1991a, b; Corrigan *et al.*, 1993; Samudralwar *et al.*, 1995; Tully *et al.*, 1995; Deibel *et al.*, 1996; Licastro *et al.*, 1996; Cornett *et al.*, 1998b; Cornett *et al.*, 1998a; Molina *et al.*, 1998; Gonzalez *et al.*, 1999). These inconsistencies are likely due to differences in methodology employed, technical difficulties encountered during tissue processing, and small sample size (Cuajungco and Faget, 2003). A consistent finding is that abnormal levels of these metals are associated with AD-affected brain areas that contain high amyloid burden (Lovell *et al.*, 1998; Suh *et al.*, 2000). It is worth noting that zinc also accumulate in the brains of APP transgenic mice, but is only present in mature, cored senile plaques (thioflavine-T staining positive), and not in diffuse or pre-amyloid plaques, suggesting that zinc may be responsible for plaque maturation (Lee *et al.*, 1999). A rather interesting observation is that when ZnT3, a neuronal vesicular zinc transporter, was knocked out ($ZnT3^{-/-}$) and crossed with the APP Tg2576 mouse transgene, the amyloid burden in brains of resulting $APP2576^{+}$: $ZnT3^{-/-}$ transgenes have significantly decreased when compared with controls (Lee *et al.*, 2002). Consequently, these mice have increased levels of soluble Aβ with no apparent adverse effects particularly on life span and at the cellular synapse (Lee *et al.*, 2002).

Like zinc levels, there are also inconsistent reports on cerebral copper levels in postmortem AD brain. Some observations show a significant decrease of copper concentrations in AD brain (Deibel *et al.*, 1996), while other studies report a 2-fold increase of copper levels in the CSF (Basun *et al.*, 1991), amyloid plaque rim (Lovell *et al.*, 1998), as well as an increase in brain and CSF ceruloplasmin levels, a known copper-binding/transporting protein (Loeffler *et al.*, 1994). There is also an indication that serum copper levels are significantly elevated among AD patients (Squitti *et al.*, 2002). It is interesting to note that *in situ*, copper ions found in plaques and tangles are redox active (Sayre *et al.*, 2000). Meanwhile, abnormal cerebral iron levels and its associated binding protein have been consistently observed in postmortem AD brain tissue (Connor *et al.*, 1992; Smith *et al.*, 1997; Lovell *et al.*, 1998; Smith *et al.*, 1998a). Iron ions present within amyloid plaques deposited in human brains (Smith *et al.*, 1997; Lovell *et al.*, 1998; Smith *et al.*, 1998b), as well as in amyloid-bearing APP transgenic mouse brains (Smith *et al.*, 1998b) are redox-active and likely to be neurotoxic (Schubert and Chevion, 1995). The implication of abnormal copper and iron levels in AD pathology is inherent to the redox properties of these two metals where

they could likely contribute to oxidative stress in the central nervous system (Halliwell, 1992, 2001), a phenomenon typically seen in brain tissues of patients with AD and animal model of the disease (Smith *et al.*, 1998b; Huang *et al.*, 1999b; White *et al.*, 1999; Huang *et al.*, 2000; Perry *et al.*, 2003).

# 3. METAL-BINDING PROPERTIES AND METAL INTERACTIONS OF AMYLOID-B AND APP

## 3.1 APP is a metalloprotein

APP has specific and saturable binding sites for zinc (APP 181-200; $K_A$ = 750 nM) (Bush *et al.*, 1993) and copper (APP 135-155; $K_D$ = 10 nM) (Hesse *et al.*, 1994). These binding sites suggest that zinc and copper serve a physiological role for APP and that these sites have homology to all known members of the APP superfamily (Bush *et al.*, 1994a; Simons *et al.*, 2002), and the amyloid precursor-like proteins 1 and 2 (Wasco *et al.*, 1992; Wasco *et al.*, 1993) giving some weight on the importance of both metals in APP function and metabolism. In fact, overexpression of APP carboxyl-terminal fragment containing Aβ, results in significantly reduced copper and iron levels in the transgenic mouse brain (Maynard *et al.*, 2002). On the other hand, APP overexpression in Tg2576 mouse transgenes results in significantly reduced levels of copper, but not iron, even prior to the appearance of amyloid neuropathology and throughout the lifespan of the mouse (Maynard *et al.*, 2002). For reasons that are unclear, an associated increase in cerebral manganese levels was observed in both transgenic strains. Nevertheless, these observations confirm the roles for APP and Aβ in physiological metal regulation.

The role of zinc on APP is believed to be in a structural capacity. For example, zinc has been shown to enhance the binding of several proteoglycans and basement membrane proteins to APP (Bush *et al.*, 1994a; Multhaup, 1994; Multhaup *et al.*, 1994; Multhaup *et al.*, 1995). Zinc also inhibits cleavage of full-length APP from human platelets (Li *et al.*, 1995). An effect that could influence the generation of Aβ from APP and may increase the biological half-life of Aβ by protecting the peptide from proteolytic attack (Bush *et al.*, 1993; Bush *et al.*, 1994a). Nevertheless, there has been no report to date which shows that zinc binding to APP results in an increase production of Aβ from APP *in vivo*. In fact, zinc (≤ 50 μM) was shown to specifically increase secreted APP, which consequently decreased the release of Aβ in the media of cultured Chinese hamster ovary (CHO) cells (Borchardt *et al.*, 2000). Like zinc, copper (10 μM) also produces a

similar effect in CHO cells where it precludes generation of Aβ by enhancing synthesis of full-length, non-amyloidogenic APP (Borchardt *et al.*, 1999). One caveat, however, is that APP can reduce Cu(II) to Cu(I) which results in oxidation of Cys144 and Cys155 and the formation of a corresponding intramolecular disulfide bridge (Multhaup *et al.*, 1996; Ruiz *et al.*, 1999). The resulting APP-Cu(I) complex is prone to redox reactions resulting in site-specific and random APP fragmentation (Multhaup *et al.*, 1998) thus increasing the possibility of pathologic production of the amyloidogenic protein.

## 3.2 Aβ is a metalloprotein and interacts with transition metals: aggregation, oxidation and cytotoxicity

The aggregational state of Aβ is spatially- and temporally-dependent, and is influenced by its concentration, by pH, and by the ionic concentrations of zinc, copper, or iron (Bush *et al.*, 1994b; Huang *et al.*, 1997; Atwood *et al.*, 1998; Huang *et al.*, 1999b; Atwood *et al.*, 2000a). Aβ1-40 contains a high affinity binding site for zinc 1:1 (zinc:Aβ; $K_D$ = 107 nM), and a 2:1 low affinity binding site ($K_D$ = 5.2 μM) (Bush *et al.*, 1993; Bush *et al.*, 1994b). The zinc binding site spans AA positions 1-16 of the Aβ protein sequence (Kozin *et al.*, 2001). Zinc and copper binding sites are mediated by histidine residues at positions 13 and 14 (Liu *et al.*, 1999; Yang *et al.*, 2000). In addition, an intramolecular histidine bridge at position 6 of Aβ peptide may also coordinate the zinc or copper ion (Curtain *et al.*, 2001).

Zinc (≥ 10 μM) binds and precipitates Aβ protein over a wide pH range (pH 6.0-8.0) (Bush *et al.*, 1994b; Brown *et al.*, 1997; Huang *et al.*, 1997). Zinc preserves the α-helical conformation of Aβ1-40 and its complexation is completely reversed by chelation (Huang *et al.*, 1997). Indeed, this observation was recently substantiated and it was also observed that zinc-Aβ complexes (ie. zinc-induced aggregates) are soluble and stable for several months (Kozin *et al.*, 2001).

Copper ions [Cu(II)] are not much better than iron, aluminum, calcium, magnesium, manganese, mercury, lead, or nickel at precipitating Aβ protein at physiological pH (pH 7.0) (Atwood *et al.*, 1998). Note, however, that Cu(II) precipitates Aβ in a reaction that is potentiated by mildly acidic (pH 6.6) conditions (Atwood *et al.*, 1998; Miura *et al.*, 2000). Copper-Aβ complexes (ie. copper-induced aggregates) have been characterized as highly structured and soluble, similar to that of zinc-Aβ aggregates (Atwood *et al.*, 1998; Miura *et al.*, 2000; Curtain *et al.*, 2001; Suzuki *et al.*, 2001). Interestingly, although Aβ binds equimolar amounts of Cu(II) and Zn(II) at pH 7.4, Cu(II) displaces Zn(II) from Zn(II):Aβ complex under mildly acidic conditions (pH 6.6) (Atwood *et al.*, 1998; Atwood *et al.*, 2000a) by

occupying the intramolecular histidine-bridge coordination sites (Curtain *et al.*, 2001). Copper seemingly competes for the zinc binding site when the Cu(II):Aβ ratio is 4, although a much lower copper concentration is needed to disrupt zinc-induced Aβ aggregation (Suzuki *et al.*, 2001). Note, however, that the stoichiometry of Cu(II):Aβ increases from zero (when Aβ is soluble) to 1.0-2.5 when Aβ is aggregated by Cu(II). Aβ1-40 has higher affinity (log $K_{app}$ 10) and lower affinity (log $K_{app}$ 7.0) binding sites for copper, but the affinity of copper for Aβ1-42 is much greater for both sites (log $K_{app}$ 16.0 and log $K_{app}$ 8.0, respectively) (Atwood *et al.*, 1998; Atwood *et al.*, 2000a). Under mildly acidic conditions (pH6.8), the affinity of Aβ1-40 and Aβ1-42 for Zn(II), but not Cu(II) decreases at the lower affinity binding sites (Atwood *et al.*, 2000a). Unlike Zn(II), both Cu(II) and Fe(III) induce greater Aβ aggregation under mildly acidic conditions (*e.g.* pH 6.6) (Atwood *et al.*, 1998). Recent studies indicate that copper's strong pH-dependence interaction with Aβ protein is due to its specific ability to bind either the N-τ of the histidine imidazole ring and main-chain amide nitrogens or N-π atom (Miura *et al.*, 2000; Suzuki *et al.*, 2001). The N-τ bridge results in copper-induced *insoluble* Aβ aggregation, while the N-π bridge produces *soluble* Aβ aggregates (Miura *et al.*, 2000; Suzuki *et al.*, 2001). Interestingly, displacement of Zn(II) by Cu(II) from Aβ's zinc binding sites supposedly inhibits zinc-induced Aβ aggregation via direct competition by Cu(II) at histidine sites. It is interesting to note that at physiological pH, zinc-induced Aβ aggregates have subtle differences from copper-induced Aβ aggregates in terms of its relative density and resolubilization state (*ie.* zinc-Aβ aggregates are denser, and harder to resolubilize than its copper-Aβ aggregates) (Moir *et al.*, 1999).

## 3.3 The neurotoxicity of Aβ is mediated by its oxidative state and redox metals

Many studies have confirmed that aged Aβ is neurotoxic *in vitro* (Pike *et al.*, 1991a, b) and *in vivo* (Emre *et al.*, 1992; Weldon *et al.*, 1998). While Aβ1-40 fragment is known to be neurotoxic (Yankner *et al.*, 1989; Pike *et al.*, 1991a, b; Kowall *et al.*, 1992), Aβ1-42 is more neurotoxic than Aβ1-40 (Huang *et al.*, 1999b), and is more likely to generate hydrogen peroxide ($H_2O_2$) than Aβ1-40 (Huang *et al.*, 1999a). In fact, the redox activity of Aβ species is greatest for Aβ1-42>Aβ1-40>>ratAβ1-40 (Huang *et al.*, 1999b). There is compelling evidence that $H_2O_2$ production is central to Aβ1-42's apparent cytotoxicity (Figure 1) (Behl *et al.*, 1994; Huang *et al.*, 1999b; Cuajungco *et al.*, 2000b). Binding of trace levels of redox active metals, Cu(II) or Fe(III), to Aβ1-42 engenders a cell-free catalytic production of $H_2O_2$ from $O_2$ via metal reduction (Huang *et al.*, 1999a; Huang *et al.*, 1999b)

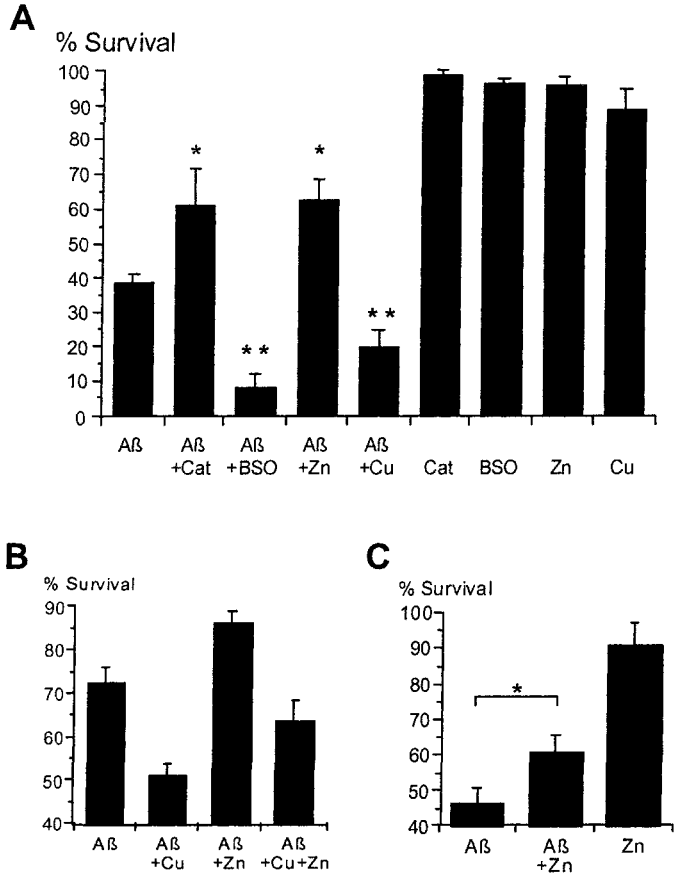


Figure 1. Effect of $Zn^{2+}$ upon Aβ1-42 cytotoxicity. *A, B.* Primary rat neuronal cultures were incubated with Aβ1-42 (*A*, 20 μM; *B*, 10 μM) and/or other factors for 48 h, and cell survival measured by Live-Dead assay, compared to untreated cultures. *A*, Effects of co-incubation with catalase (Aβ+Cat), buthionine sulfoximine (Aβ+BSO), $Zn^{2+}$ (20 μM, Aβ+Zn), $Cu^{2+}$ (20 μM, Aβ+Cu), incubated and effects of these factors alone, upon neuronal survival are shown. *C*, human embryonic kidney 293 cells were with Aβ1-42 (10 μM) ± $Zn^{2+}$ (10 μM) or $Zn^{2+}$ alone, as shown. Surviving cells were assayed, compared to untreated control cultures. Data are represented as mean ± SEM, $N$ = 5-6 experimental trials performed in triplicate wells (* $p < 0.01$, ** $p < 0.001$). Reprinted with permission, Cuajungco *et al.* 2000, *J. Biol. Chem.*, **275**:19439-19442. Copyright 2000, The American Society for Biochemistry and Molecular Biology, Inc.

and can be inhibited by high concentrations of zinc (Figure 2) (Cuajungco *et al.*, 2000b). On the other hand, copper competes with zinc, and is believed to augment the oxidation of Aβ which could affect its metabolism (Atwood *et al.*, 2000b) and neurotoxicity (Huang *et al.*, 1999b; Cuajungco *et al.*, 2000b).

Aβ's conformational state mediates its intrinsic toxicity and oxidative properties in the presence of redox active metals (Pike *et al.*, 1991a, b; Lorenzo and Yankner, 1994; Huang *et al.*, 1999b; Atwood *et al.*, 2000b; Cuajungco *et al.*, 2000b; Monji *et al.*, 2001, 2002). In fact, it was observed recently that the oxidative cytotoxicity of Aβ is caused by pre-fibrillar structure and not fibrillar Aβ, at least in a specific *C. elegans* strain (Drake *et al.*, 2003).

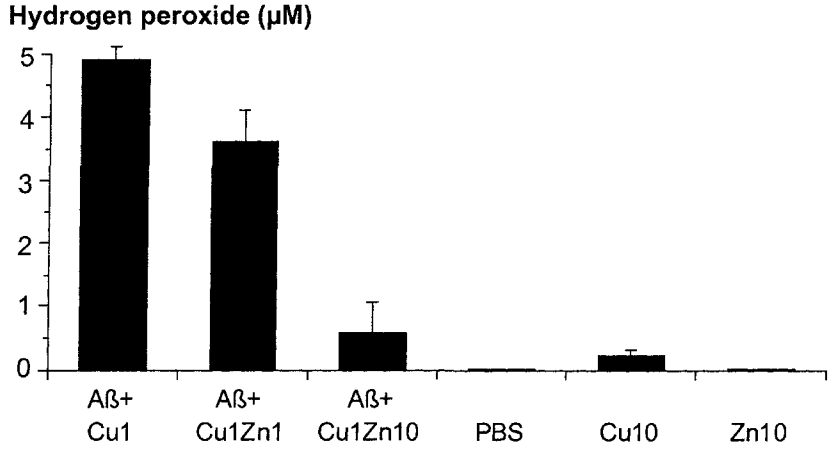


Figure 2. Effect of $Zn^{2+}$ on cell-free $H_2O_2$ production by Aβ1-42. Aβ1-42 peptide (10 μM) was incubated for 1 h at 37°C in PBS with $CuCl_2$ (Cu1 = 1 μM), ± $ZnCl_2$ (Zn1 = 1 μM, Zn10 = 10 μM) and levels of $H_2O_2$ measured. The background levels of $H_2O_2$ production in the absence of peptide were also measured. Data are means ± SEM ($N$ = 5 experimental trials performed in triplicate wells). Reprinted with permission, Cuajungco *et al.* 2000, *J. Biol. Chem.*, **275**:19439-19442. Copyright 2000, The American Society for Biochemistry and Molecular Biology, Inc.

Nevertheless, the biochemical relationship between Aβ's toxicity and oxidative stress in AD is rather complex and not well understood. Studies indicate that cerebral Aβ deposition and oxidative stress are considered closely related to the etiology of AD. Indeed, metabolic signs of oxidative stress such as oxygen radical-mediated damage of brain proteins, lipids and nucleic acids, as well as systemic signs of oxidative stress and the response of antioxidant systems have all been observed in AD tissue (Mecocci *et al.*, 1994; Smith *et al.*, 1997; Smith *et al.*, 1998b; Mecocci *et al.*, 2002). Likewise, Aβ induces lipid peroxidation (Butterfield *et al.*, 1999a; Butterfield *et al.*, 2002a; Butterfield *et al.*, 2002b; Butterfield and Lauderback, 2002) and exerts toxicity through mechanisms involving the generation of cellular $H_2O_2$ through redox metal interactions (Behl *et al.*,

1994; Huang *et al.*, 1999b; Cuajungco *et al.*, 2000b). This toxic effect is abolished by superoxide/$H_2O_2$ scavengers, antioxidants, and metal chelators (Schubert and Chevion, 1995; Bruce *et al.*, 1996; Butterfield *et al.*, 1999b; Sayre *et al.*, 2000; Rottkamp *et al.*, 2001). In a clinical setting, there is evidence that treatment of patients suffering from AD with an antioxidant (vitamin E) delays their cognitive decline (Sano *et al.*, 1997). Taken together, it seems that the oxidative stress in AD occurs via elevated $H_2O_2$ levels and promote cytotoxicity by incessantly taxing cellular antioxidant system, and by interacting with transition metals that could produce the highly toxic hydroxyl radicals (Halliwell, 1992, 2001). These reports reinforce the belief that the pathologic basis of AD is through oxidative stress mitigated by abnormal metabolisms of Aβ and its interaction with redox active metals.

# 4. METAL CHELATORS: THERAPEUTIC POTENTIAL FOR ALZHEIMER'S DISEASE

## 4.1 Basic science studies: chelator-induced neutralization of Aβ-mediated oxidative activity and resolubilization of amyloid plaques

Aβ reduces Cu(II) and Fe(III) to Cu(I) and Fe(II), respectively, and that Aβ can generate $H_2O_2$ through a metal-dependent reaction (Huang *et al.*, 1999a; Huang *et al.*, 1999b; Cuajungco *et al.*, 2000b). Henceforth, using metal chelating agents to disrupt this interaction with redox active metals and oxidative activity seems to be the most promising and appropriate therapy for AD. Indeed, Huang *et al.* previously reported that the metal chelators, bathophenantroline disulfonic acid (BP) and diethylenetetraamine pentaacetic acid (DTPA), significantly hindered with the redox activity of Aβ by quenching the $H_2O_2$ produced through Aβ-metal interactions (Huang *et al.*, 1999b). Furthermore, desferrioxamine (DFO; Fe[III]-, and Cu[II]-selective), and DTPA (Fe[III]-, Fe[II], Cu[II]- and Zn[II]-selective) have been found to neutralize Aβ-metal-generated $H_2O_2$ *in situ* (Sayre *et al.*, 2000). Note that when Aβ is pretreated with DFO, its neurotoxicity *in vitro* is abolished (Rottkamp *et al.*, 2001).

To assess the potential of metal chelating agents to abstract metals from Aβ and solubilize the protein from senile plaques, Cherny *et al.* used N,N,N',N'-tetrakis-(2-pyridylmethyl)-ethylenediamine (TPEN), bathocuproine disulfonic acid (BC), and ethylene glycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) and successfully showed an

enhancement in resolubilization of Aβ deposits from post-mortem AD and non-AD brain samples (Figure 3) (Cherny *et al.*, 1999). Likewise, using either triethylenetetraamine (TETA; a high affinity Cu[II] chelator) or bicinchoninic acid (BCA; a Cu[I]-selective chelator), marked increase in resolubilization of Aβ deposits from brain samples of APP Tg2576 was also observed (Gray *et al.*, 1998). This led Cherny *et al.* to perform a pilot blind study using clioquinol (iodochlorhydroxyquin, CQ; a hydrophobic drug with metal chelating property that crosses the blood-brain barrier) as an oral therapy for 21-month old APP Tg2576 transgenic mice (Cherny *et al.*, 2001). The group found that after 9 weeks, a 49% decrease in brain Aβ deposition (-375 μg/g wet weight, $p = 0.0001$) was observed. In addition to the lowering of cerebral amyloid burden, there was a modest increase in soluble Aβ (1.45% of total cerebral Aβ), while the levels of APP, synaptophysin, and glial fibrillary acidic protein (GFAP) were not significantly affected. It appeared that the animals showed an overall increase in weight, general health, motor activity, and alertness relative to untreated controls; however, these improvements did not affect the lifespan of these animals.

While the above results show some promise on chelation therapy, one caveat for using metal chelators in the brain is that it has been reported that certain chelating agents, regardless of their affinity or specificity for a particular brain metal, must be used with caution since distinct compounds can produce severe side effects that could lead to seizure and significant neuronal death when administered directly into the brain (Cuajungco and Lees, 1996, 1998; Lees *et al.*, 1998; Armstrong *et al.*, 2001).

## 4.2 Clinical studies: past and present

The principle of a therapeutic drug abstracting a metal on a protein target is well-developed in pharmacology. A number of well-known antibiotic, anticonvulsive, antitumor, and antiinflammatory drugs exert their pharmacological effect by interacting with the Cu-, Zn- or Fe-active sites of their target protein (Cuajungco *et al.*, 2000a). Historically, the use of the iron chelator, DFO, in a clinical setting was the first published attempt as therapeutics for AD. DFO proved to have some positive effects on the rate of cognitive decline among AD patients (Crapper McLachlan *et al.*, 1991).

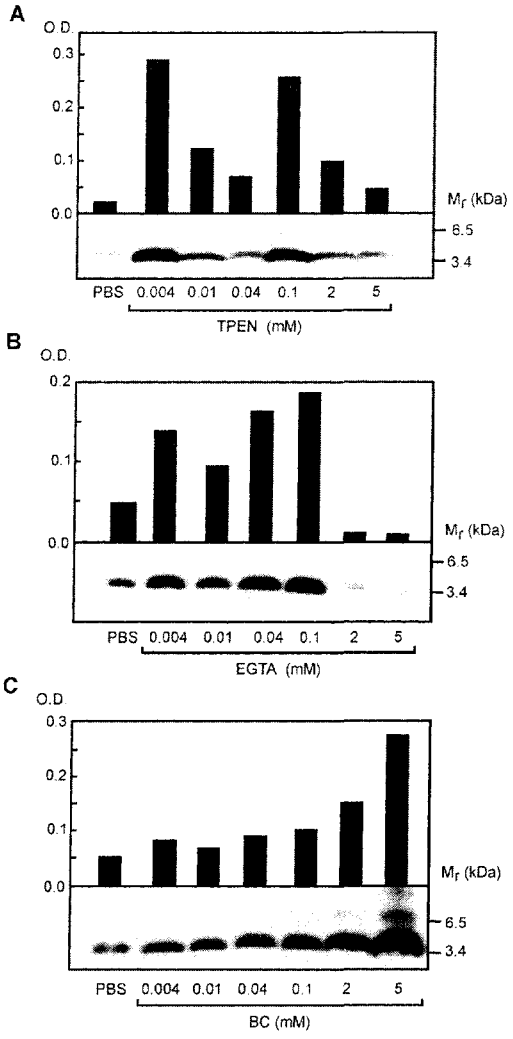


Figure 3. Release of Aβ from sedimentable deposits by chelators. Frontal cortex from an AD brain was homogenized in PBS, pH 7.4, with or without increasing concentrations of TPEN (*A*), EGTA (*B*), or BC (*C*). Following centrifugation, Aβ in the supernatants was visualized by Western blot using anti-Aβ monoclonal antibody WO2 (*lower panels*), and quantified by densitometry (*graphs* above corresponding blots). Although there is considerable variation in the optimum chelator concentration for the maximal recovery of Aβ from case to case, these data are representative of 17 AD cases. Reprinted with permission, Cherny *et al.* 1999, *J. Biol. Chem.*, **274**:23223-23228. Copyright 1999, The American Society for Biochemistry and Molecular Biology, Inc.

There was, however, no follow-up study done to verify this result. DFO, while it binds iron with high avidity, also chelates zinc and copper (Cuajungco *et al.*, 2000a; Cuajungco and Faget, 2003). CQ, a USP antibiotic with metal chelating properties, shows some promise for clinical use in AD as current reports showed that the drug appears to be well-tolerated in mice when administered orally (Cherny *et al.*, 2001; Nitzan *et al.*, 2003). Recently, a short-term Swedish study on the efficacy of CQ on 10 AD patients (80 mg/day) resulted in modest cognitive improvement after 21 days (Regland *et al.*, 2001). Because this was a very short study with small patient number, it was hard to conclude if CQ was really effective. More recently, though, Ritchie *et al.* reported a more promising result on the use of CQ for AD (Ritchie *et al.*, 2003). The effect of CQ treatment was significant in the more severely affected group with a baseline cognitive subscale score of greater than or equal to 25 using the Alzheimer's Disease Assessment Scale (ADAS). The placebo group had substantial worsening on ADAS scores when compared with minimal deterioration for the CQ-treated group. It is noteworthy that measurement of plasma Aβ1-42 levels declined in the CQ-treated group while the placebo group showed an increase. While the drug was well tolerated, plasma zinc levels rose in the CQ-treated group with no apparent ill effects. It was argued that while the sample size in this pilot Phase 2 clinical study was small, the results warrant further investigation on this novel treatment strategy for AD.

## 5. CONCLUDING REMARKS

Metal chelation therapy for AD is still in its infancy. Recent observations indicate that an ideal chelating drug to dissolve Aβ would involve a molecule that avidly binds and is relatively selective for Cu(I), Zn(II) and possibly Fe(III), but does not sequester other abundant, yet crucial cations like calcium and magnesium. Electrically neutral and non-polar molecules are ideal chelators, since they are best absorbed across the gastrointestinal tract and achieve a broad distribution throughout various tissues. Abstraction of metals from protein still has many obstacles due to the potential difficulties in: (a) route of administration; (b) reaching the specific target brain tissue due to protection by the blood-brain barrier; (c) possible associated non-specific problems of systemic metal ion depletion; and (d) potential severe side effects by the drug itself. Further investigations on the molecular and physiological effects of metal chelating agents are necessary to circumvent these potentially severe complications. Notwithstanding, can we ask the question therefore whether metal chelators will be pharmacologically useful for Alzheimer's disease? The answer to this question remains uncertain, although very encouraging on the bases of

several pilot Phase 2 clinical trials of clioquinol. More clinical investigations to establish unequivocal drug efficacy of clioquinol and other candidate metal chelators for patients with AD are clearly warranted.

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