

Current Status of Metals as Therapeutic Targets in Alzheimer's Disease

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There is accumulating evidence that interactions between β -amyloid and copper, iron, and zinc are associated with the pathophysiology of Alzheimer's disease (AD). A significant dyshomeostasis of copper, iron, and zinc has been detected, and the mismanagement of these metals induces β -amyloid precipitation and neurotoxicity. Chelating agents offer a potential therapeutic solution to the neurotoxicity induced by copper and iron dyshomeostasis. Currently, the copper and zinc chelating agent clioquinol represents a potential therapeutic route that may not only inhibit β -amyloid neurotoxicity, but may also reverse the accumulation of neocortical β -amyloid. A Phase II double-blind clinical trial of clioquinol with B₁₂ supplementation will be published soon, and the results are promising. This article summarizes the role of transition metals in amyloidogenesis and reviews the potential promise of chelation therapy as a treatment for AD. *J Am Geriatr Soc* 51:1143–1148, 2003.

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In recent years, β -amyloid has been implicated as the target protein in the pathogenesis of Alzheimer's disease (AD). Dense extracellular deposits of β -amyloid, a pathological hallmark of AD, collect as masses of twisted β -pleated sheet fibrils outside of the cell bodies of neurons and glia (senile plaques) and within cerebral blood vessels (amyloid congophilic angiopathy).^{1,2} The amyloid cascade theory has led to several therapeutic approaches that specifically target β -amyloid. The first approach, the secretase inhibitors, focuses on inhibiting the generation of β -amyloid from its parent protein, the amyloid precursor protein.^{3,4}

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The second approach, the β -amyloid vaccine, focuses on clearing all β -amyloid from the brain with specific antibodies.⁵ A third and very different methodology involves treatment with metal complexing agents. Treatment with metal complexing agents focuses on abstracting copper, iron, and zinc, which may be responsible for inducing the neurotoxic action of β -amyloid. It is currently hypothesized that age-associated abnormalities of copper, iron, and zinc homeostasis induce the aggregation and toxicity of β -amyloid.⁶ The thesis of this paper will address the case for and potential of metal complexing agents. For comparison, the three primary therapeutic approaches are summarized in Table 1.

β -AMYLOID

There are several species of β -amyloid. The species differ in their primary structure, which ranges from 39 to 43 amino acids. The 40-amino acid β -amyloid (A β 1–40) is the primary free soluble species found in biological fluids, whereas the 42-amino acid β -amyloid (A β 1–42) is the primary component of β -amyloid plaques.^{7,8} Soluble diffuse β -amyloid is considered to be a healthy component of cerebrospinal fluid, but under atypical conditions, β -amyloid is induced to aggregate and produce oxidative stress.⁹ Evidence for induced β -amyloid toxicity is found in the region specific for deposition of amyloid.¹⁰ β -amyloid is a ubiquitously expressed protein, whereas deposits of amyloid are primarily found in relation to synapses and the cerebrovascular lamina media (reviewed in ¹⁰). A further example is found in the physiology of familial Alzheimer's disease and Down's syndrome.¹¹ Familial Alzheimer's disease and Down's syndrome manifest in the overexpression of the A β 1–42 over a lifespan,¹¹ but it is only in early adulthood that the disease becomes evident.¹¹ The dys-homeostasis of copper, iron, and zinc is currently hypothesized to be an age-dependent factor that induces β -amyloid neurotoxicity. Evidence for a significant metal dyshomeostasis is highlighted in Table 2.^{12–14}

COPPER

Copper is thought to interact with β -amyloid in two ways significant to AD pathology. First, copper mediates the aggregation of β -amyloid under slightly acidic conditions. Second and more importantly, copper serves as a cofactor to β -amyloid, facilitating the generation of oxidative stress.

Table 1. Potential Anti-Amyloid Therapeutic Approaches to Alzheimer's Disease

Investigational Agent	Putative Mechanism of Action	Evidence	Status	Potential Areas of Concern
Secretase inhibitors ^{3,4}	Prevents synthesis of β -amyloid. Inhibits cleavage of β -amyloid from the parent protein amyloid precursor protein (APP).	A functional γ -secretase inhibitor was found to reduce to the concentration of β -amyloid in a dose-dependent manner in a study of mice transgenic for human APP.	Bristol Myers Squibb described a clinical trial of γ -secretase inhibitor as well tolerated in humans (one dose) (K. Fehlsenstein). Details are unknown.	<ol style="list-style-type: none"> 1. Inhibitors may have multiple substrates. 2. Physiological purpose of β-amyloid is unknown. Eliminating a protein whose function is unknown poses a physiological threat.
Amyloid vaccine ⁵	Immunization with aggregated β -amyloid induces antibody response. Elicited antibody binds to and facilitates the clearance of β -amyloid.	Brain β -amyloid decreased 60% in a blinded 4-month study of 15-month-old transgenic mice. Brain β -amyloid decreased 80% in a blinded 7-month study of 18-month-old transgenic mice.	AN-1792 amyloid vaccine phase IIA trial suspended. Fifteen of 360 patients developed symptoms of central nervous system inflammation. Passive immunity approach under investigation.	<ol style="list-style-type: none"> 1. Irreversible complications of the central nervous system. 2. Autoimmune complications resulting from humans being vaccinated with their own protein. 3. Physiological purpose of β-amyloid is unknown. Eliminating a protein whose function is unknown poses a physiological threat.
Metal complexing agents ^{6,35} (MCA)	Abstracts copper, iron, and zinc from β -amyloid plaques. Inhibits copper and iron-dependent neurotoxicity. Facilitates amyloid plaque disaggregation.	Brain β -amyloid decreased 50% in a blinded, randomized 9-week study of 21-month-old transgenic mice and clioquinol (orally administered MCAs).	Clioquinol Phase II trial completed. Results pending and promising.	<p>MCAs may deplete biomaterials. Solubilizing the amyloid plaques increases the concentration of soluble β-amyloid, a neurotoxic species.</p>

Note: This table represents potential Alzheimer's disease therapeutics in Phase II clinical trials
MCA = metal complexing agent.

Table 2. Evidence for a Metal Dyshomeostasis in Alzheimer's Disease (AD)

Study	Copper	Iron	Zinc
Copper, iron, and zinc levels in human neuropil* and senile plaques determined by microparticle-induced x-ray emission ¹²	Total amyloid plaque: 25 $\mu\text{g/g}$ AD neuropil: 19 $\mu\text{g/g}$ Control neuropil: 4 $\mu\text{g/g}$	Total amyloid: 53 $\mu\text{g/g}$ AD neuropil: 39 $\mu\text{g/g}$ Control neuropil: 19 $\mu\text{g/g}$	Total amyloid plaque: 69 $\mu\text{g/g}$ AD neuropil: 51 $\mu\text{g/g}$ Control neuropil: 23 $\mu\text{g/g}$
Abnormal zinc deposits in human AD brain found with immunohistochemical methods ¹³	NA	NA	Senile plaque amyloid stained positive for zinc; nondemented brains rarely stained
Reactive zinc in rat brain transgenic for AD detected via immunohistochemical methods ¹⁴	NA	NA	Found significant concentrations of chelatable zinc present in mature congophilic plaques

*Neuropil is the matter outside of the cell bodies of neurons and glia (vessels).

NA = not applicable.

A preclinical study of synthetic β -amyloid found that submicromolar concentrations of copper induced the aggregation of low nanomolar concentrations of β -amyloid under slightly acidic conditions (hydrogen ion concentration (pH) 6.6–7.0) in vitro.⁹ The study also found that the aggregating effects of copper were reversible upon addition of a copper-chelating agent or with an increase in pH.⁹ In a postmortem AD study, chelating agents were evaluated for their ability to induce the solubility of β -amyloid.¹⁵ It was found that chelating agents at concentrations as low as 4 μM were effective at abstracting metal ions and resolubilizing β -amyloid.¹⁵

Of greater significance is a study that established that synthetic β -amyloid in the presence of a catalytic quantity of copper produces hydrogen peroxide (H_2O_2).⁹ Several studies have shown that β -amyloid facilitates apoptosis in neuronal cell cultures.^{16–18} Therefore, evidence for the generation of H_2O_2 via a β -amyloid–copper complex establishes a possible mechanism for β -amyloid-mediated neurotoxicity.¹⁹ Using spectrophotometric experiments, it was shown that β -amyloid binds to and reduces copper²⁺ to copper¹⁺, followed by formation of H_2O_2 by double electron transfer to oxygen (O_2).¹⁹ (These reactions took place in a cell-free environment and are O_2 dependent.¹⁹) Toxicity due to the generation of H_2O_2 was confirmed by the addition of catalase, an enzyme that converts H_2O_2 to water.¹⁵ Catalase decreased β -amyloid toxicity by 75%.¹⁵

The synthesis of H_2O_2 via the β -amyloid–copper complex is a highly probable reaction in vivo.¹⁵ The copper $\text{A}\beta$ 1–42 complex has a significant positive reduction potential ($\approx +500$ – 550 mV vs silver/silver chloride) that is analogous to strongly reducing cuproproteins and oxidases.²⁰ In addition, the copper β -amyloid dissociation constants (K_d) are significant. The low-affinity binding (K_d) is approximately $5.0 \times 10^{-9}\text{M}$, whereas the $\text{A}\beta$ 1–42 high-affinity binding (K_d) is $7.0 \times 10^{-18}\text{M}$.²¹ In comparison, the $\text{A}\beta$ 1–40 high affinity binding (K_d) is $5.0 \times 10^{-11}\text{M}$.²¹ The greater affinity of $\text{A}\beta$ 1–42 for copper compared with that of $\text{A}\beta$ 1–40 accounts for the preferential precipitation of $\text{A}\beta$ 1–42.²¹

H_2O_2 is inherently deleterious to cells.¹⁵ It can easily diffuse, permeate a cell membrane, and induce apoptosis.¹⁵

In addition, the generation of H_2O_2 and copper¹⁺ creates conditions ideal for the generation of highly reactive hydroxyl radicals (via Fenton-type chemistry).²² When unregulated, the hydroxyl radical can initiate lipid peroxidation, protein modifications such as cross-linking and carbonylation, and damage to deoxyribonucleic acid through strand breaks, modified bases, and cross links.²³ Studies have shown that β -amyloid serves as a substrate for the hydroxyl radical.²¹ β -amyloid extracted from postmortem AD brains has shown functional group modifications such as carbonyl adduct formation, histidine loss, and di-tyrosine cross-linking.²¹ These modifications make the protein resistant to protease degradation and decrease its solubility.²¹

IRON

Iron is a redox active metal, and its contributions to AD pathology are thought to be similar to those of copper. In vitro synthetic β -amyloid studies have found that iron induces the aggregation and potentiates the neurotoxicity of β -amyloid.²⁴ β -amyloid binds to and reduces iron³⁺ to iron²⁺, which is followed by formation of H_2O_2 via mechanisms similar to those described for copper.²⁴ The generation of iron²⁺ and H_2O_2 creates conditions ideal for the Fenton reaction, which leads to the formation of highly reactive hydroxyl radicals.²⁴ Iron precipitates β -amyloid to a lesser degree than does copper and catalyzes the Fenton reaction at a significantly slower rate.¹⁹ Although iron does not contribute to β -amyloid neurotoxicity to the same degree as copper, it is also hypothesized to be a significant participant in the pathogenesis of AD.

ZINC

Zinc has been found to be markedly elevated in amyloid plaques, but unlike copper and iron, zinc is a redox-inert metal. Zinc in the AD brain functions to precipitate β -amyloid and inhibit β -amyloid production of H_2O_2 to a limited degree.

In a study of synthetic β -amyloid, micromolar concentrations of zinc were found to rapidly precipitate β -amyloid at physiological pH (7.4), unlike copper and iron, which precipitate β -amyloid under mildly acidic conditions.⁹ Zinc-induced β -amyloid precipitation was denser and less

easily resolubilized.²⁵ It is thought that zinc's tendency to precipitate β -amyloid at physiological pH inhibits β -amyloid plaque clearance and degradation.

Several studies have contributed to the hypothesis that, although copper and iron facilitate β -amyloid toxicity, elevations in interstitial zinc reflect an antioxidant response.²⁶ Evidence for zinc's inhibitory role was found in a negative correlation between amyloid burden and levels of 8-hydroxyguanosine, a marker of hydroxyl radical activity.²⁶ A study of β -amyloid, copper, and zinc determined that zinc inhibits H_2O_2 production from A β 1–42 by competing with copper for active sites on β -amyloid.²⁶ Zinc in a 1:1:1 coinubation with copper and A β 1–42 was found to inhibit H_2O_2 production by 25%.²⁶ Zinc in a 10:1:1 coinubation with copper and A β 1–42 was found to inhibit H_2O_2 production by 90%.²⁶ Therefore, although zinc appears to degrade the neurotoxic activity of β -amyloid, it is not an efficient mechanism for eliminating neurotoxicity. A further study found that plaque β -amyloid is not loaded with the concentration of zinc necessary to completely neutralize the neurotoxic activity of β -amyloid.²⁷ Therefore, the antioxidant properties of zinc may not be an effective means of preventing β -amyloid neurotoxicity.

Several mechanisms have been proposed to account for the significant concentrations of zinc in relation to neurotoxic β -amyloid. Zinc may be released from metallothionein thiols in response to H_2O_2 generation,²⁸ or glial activation may release zinc from the metallothionein pool.²⁹ Additionally, a parallel between areas of β -amyloid deposits and areas of loosely bound zinc, which is most concentrated in the corticofugal system, has been established.³⁰

Further support for zinc's contribution to AD pathology was found in a recent experiment, which crossed a mouse model of AD with a knockout mouse lacking the zinc transport protein (ZnT3).³¹ The ZnT3 loads synaptic zinc and accounts for 20% to 30% of total brain zinc.³¹ The resultant mouse model of AD lacking the zinc transport protein was found to have markedly diminished concentrations of cerebral β -amyloid plaques.³¹

POTENTIAL THERAPEUTICS

Nutritional Implications

Brain function depends on high concentrations of metals; as a result, numerous mechanisms exist for their regulation, transportation, and compartmentalization.³² For example, at neurotransmission, approximately 300 μ M of zinc is released. In neuronal cell culture, 300 μ M of zinc would be rapidly toxic.³⁰ The significant concentrations of metals stored in the brain are enough to damage or dysregulate numerous proteins and metabolic systems, but the brain contains efficient homeostatic mechanisms and buffers to prevent abnormal uptake and compartmentalization.³² In addition, the blood–brain barrier is relatively impermeable to changes in the concentrations of plasma metal ions.³² Abnormal metal-protein interactions *in vivo* are most probably due to altered copper, zinc, and iron metabolism in the brain but not toxic exposure.³² The results of the zinc ablation study support this theory. The mouse model of AD, which lacked the zinc transport protein ZnT3, was found to have a marked decrease in the concentrations of cerebral β -amyloid plaques. This study sup-

ports the theory that the homeostasis of endogenous metals is disrupted, which leads to amyloid deposition.³¹ In addition, areas of histochemically reactive zinc in synaptic vesicles correlate with the regional distribution of plaques specifically in the olfactory bulb, cerebral cortex, and limbic area. The lack of zinc in the cerebellum correlates with a lack of amyloid plaques.³¹ Therefore, it is hypothesized that a mismanagement of endogenous metals, as opposed to toxicological exposure, contributes to the pathogenesis of AD.

Chelating Agents

A chelating agent is a molecule capable of binding to a metal ion. Chelating agents compete with and in some cases prevent ligands in the body from binding with metal ions (e.g., β -amyloid). Chelating agents have the potential to inhibit the copper-, iron-, and zinc-dependent neurotoxicity in the AD brain. Two such agents, desferrioxamine (DFO) and clioquinol (CQ), have been evaluated with relative success.

Desferrioxamine

In a paper published in 1991, the effects of DFO in slowing the progression of AD was reported.³³ DFO is a chelating agent that was chosen for its ability to bind aluminum (III oxidation state).³³ At the time, aluminum was hypothesized to contribute to AD pathology. The trial was a 2-year placebo-controlled, single-blind study of 48 patients with moderate AD.³³ Subjects were randomized to receive DFO (125 mg intramuscularly twice daily, 5 days a week, for 24 months), oral placebo (lecithin), or no treatment.³³ The primary efficacy measure, the video recorder home-behavioral assessment, consisted of 48 activities of daily living (ADLs) and was assessed at 6, 12, 18, and 24 months.³³ The results from the oral placebo (lecithin) group did not statistically differ from the no-treatment group, so the data were combined into a no-treatment group.³³

The results of the study were significant. It was found that the no-treatment group declined in ADLs at twice the rate of the DFO treatment group.³³ Although current evidence does not support a major role for aluminum in the pathogenesis of AD, another study suggested that the beneficial effect of the treatment was due to chelation of iron, copper, and zinc.¹⁰ This is probable, given that DFO is a relatively nonspecific chelating agent that can also bind copper, iron, and zinc.¹⁰ It was later verbally reported at the International Conference on Alzheimer's Disease, Padua, Italy, 1992, that zinc and iron concentrations were decreased in a postmortem analysis of DFO-treated subjects.¹⁰

Although DFO was reported to significantly affect the progression of AD, it is not an optimal therapeutic agent. The administration of DFO requires painful intramuscular injections twice a day and can result in problems of systemic metal ion depletion (anemia).¹⁰ DFO is a relatively unstable molecule that decomposes quickly after administration.³⁴ In addition, DFO is a polar molecule, which inhibits its ability to cross the blood–brain barrier to any significant degree.³⁴ Currently, researchers are investigating a more optimal chelating agent, CQ.

Clioquinol

In response to evidence that metal– β -amyloid interactions are the basis of AD pathophysiology, chelating agents have

been investigated for their potential to dissolve β -amyloid aggregations and inhibit H_2O_2 formation. A quinoline, 5-chloro-7-iodo-8-hydroxyquinoline (CQ), was identified as an optimal therapeutic agent for AD pathophysiology. CQ is an established U.S. pharmacopeia antibiotic with selective affinities for copper and zinc.³⁵ Most importantly, CQ is a hydrophobic molecule that can cross the blood–brain barrier and penetrate β -amyloid plaques.³⁶

Initial trials of CQ tested its ability to dissolve β -amyloid aggregates from postmortem tissue of human patients with AD. CQ was found to dissolve β -amyloid in a concentration-dependent manner, with more than 200% β -amyloid liberated in the presence of 0.4 μ M CQ or more.³⁵ Subsequent extractions of the initial sample liberated approximately the same amount of β -amyloid.³⁵ This procedure was successfully repeated until the majority of the β -amyloid was made soluble.³⁵

Two trials of CQ were then run in transgenic mice. Cerebral β -amyloid deposition does not naturally occur in mice, but a transgenic mouse model has been developed that exhibits Alzheimer's-type neuropathology.³⁷ The first trial was a 12-week placebo-controlled, blinded study of 15-month-old transgenic mice.³⁵ CQ was administered daily at 20 mg/kg by gavage.³⁵ The results showed that five CQ-treated mice had a 65% decrease in sedimentable β -amyloid.³⁵ Two CQ-treated mice had no measurable β -amyloid in the pellet fraction and no detectable β -amyloid pathology by immunocytochemistry.³⁵

The second, more-extensive, trial was a 9-week placebo-controlled, blinded study of 21-month-old transgenic mice.³⁴ CQ was administered daily at 30 mg/kg by gavage.³⁵ The results were a 49% decrease in sedimentable β -amyloid and a decrease in the immunohistochemical amyloid plaque surface area.³⁵ There were no adverse events, and no depletion of metals from the brain or peripheral tissues were detected.³⁵ Depletion of metals from the brain or peripheral tissues is a potential area of concern and must be monitored carefully in future studies. A blinded operator assessed gross physiological changes daily using a five-point integer scale that subjectively rated a combination of general features (motor activity, alertness, and general health signs).³⁵ A significant gross physiological improvement in the CQ-treated mice was detected and maintained after 16 days of treatment.³⁵

In addition, the second trial found elevated concentrations of soluble β -amyloid in the CQ-treated mice. This was a 50% increase over that of the control group but only a 1% rise in total β -amyloid levels.³⁵ Elevated concentrations of soluble β -amyloid are a potential source of concern. Three independent studies have found a positive correlation between degree of disease severity and elevated concentrations of soluble β -amyloid.^{2,38,39} These studies support the theory that a form of soluble β -amyloid is neurotoxic. It was reported that there was no evidence that any adverse effects, abbreviated life span, or synaptic loss accompanied the increase in soluble β -amyloid.³⁵ Another report found no apparent detrimental effects when the soluble levels of β -amyloid markedly increased in the resultant mouse model of AD lacking the zinc transport protein.³¹ One group hypothesized that liberated β -amyloid was a nontoxic form or was a toxic form neutralized by a reaction with CQ,³⁵ but further studies with cognitive and

behavioral assessments are needed to assess the effects of elevated concentrations of soluble β -amyloid post-CQ treatment. There is concern over the potential return of CQ.⁴⁰ CQ had been used extensively as an antibiotic for more than 20 years when its production ended in the early 1970s,³⁵ when it was withdrawn because it became associated with subacute myelo-optic neuropathy (SMON), an uncommon neurological syndrome that primarily occurred in Japan.³⁵ At the time, a causal relationship between CQ and SMON was assumed but not developed.³⁵ Currently, it is thought that demographic factors prevalent in Japan made the population more susceptible to developing SMON.³⁵ Through 1975, there were 10,000 cases of SMON in Japan and only 220 cases reported in the rest of the world.³⁵ Twenty-five percent of the cases with SMON had never taken CQ.³⁵

SMON resembles an accelerated form of subacute combined degeneration due to vitamin B_{12} deficiency.³⁵ It has been proposed that the Japanese were endemically B_{12} deficient as a consequence of their postwar diet and were suffering from subacute combined degeneration.³⁵ Japanese suffering from B_{12} deficiency presented with symptoms of abdominal pain and diarrhea and were subsequently treated with the CQ.³⁵ Unfortunately, treatment with CQ compounded their state of B_{12} deficiency. Recently, it was found that mice treated with CQ have depleted concentrations of vitamin B_{12} in the brain and serum.⁴¹ Although the history of CQ in Japan is unclear, every precaution in clinical trials of CQ should be taken.

Recently, one study reported completion of a Phase I clinical trial of CQ with B_{12} supplementation.⁴² The trial was a 21-day open-label study of 20 patients with a diagnosis of probable AD. The patients were randomized to receive 20 or 80 mg CQ a day. Every patient received 1 mg of cyanocobalamin and 5 mg of folic acid a day.⁴² No adverse systemic or neurological events were reported.⁴² Slight improvements in cognition were found.⁴² In particular, both treatment groups improved slightly (2.7 points) on the AD Assessment Scale—cognitive subscale (ADAS-cog) ($P < .07$) and on the ADAS-cog naming, instructions, and comprehension subtests ($P < .05$).⁴² The high-dose treatment group showed improvement on ADAS-cog comprehension ($P < .05$), whereas the low-dose treatment group did not.⁴² No improvement on the Mini-Mental State Examination was found.⁴²

The results of a promising Phase II double-blind clinical trial of CQ with B_{12} supplementation in patients with AD will be published soon.⁴³ The results of this study will further determine what potential CQ might have as a therapeutic for AD, but although the results of the CQ trials are exciting, CQ remains an investigational agent, and further larger-scale safety and efficacy studies are necessary.

CONCLUSION

The last several years have witnessed rapid advances in understanding of the mechanisms that induce oxidative stress in the AD brain. The interactions of β -amyloid and mismanaged copper and iron are likely to be critical in contributing to the source of oxidative stress in AD. Zinc, which is normally enriched in the neocortical areas of the AD brain, represents a potential antioxidant response and acts to precipitate and quench β -amyloid activity. Experi-

mental studies provide strong evidence that chelation strategies targeting these metals may have the potential to dissolve β -amyloid aggregations and inhibit H_2O_2 formation. Clinical trials are ongoing to test this hypothesis. The metallobiology of β -amyloid represents a significant advance in understanding AD pathophysiology. Pharmacological interdiction of abnormal β -amyloid/metal interactions holds great promise for the development of new drugs for AD.

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