Possible Involvement of Copper(II) in Alzheimer Disease

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The β-amyloid (Aβ) peptide is a principal component of insoluble amyloid plaques that are characteristic neuropathological features of Alzheimer disease (AD). The amyloid peptide also exists as a normal soluble protein that undergoes a pathogenic transition to an aggregated, fibrous form. This transition can be affected by extraneous proteinaceous elements and nonproteinaceous elements such as copper ions, which may promote aggregation and/or stabilization of the fibrils. Copper has been found in abnormally high concentrations in amyloid plaques and AD-affected neuropil, and copper-selective chelators have been shown to dissolve Aβ peptide from postmortem brain specimens. Although Cu^{2+} is an essential element for life and the function of numerous enzymes is basic to neurobiology, free or incorrectly bound Cu^{2+} can also catalyze generation of the most damaging radicals, such as hydroxyl radical, giving a chemical modification of the protein, alternations in protein structure and solubility, and oxidative damage to surrounding tissue. ***Key words:*** **Alzheimer disease, β-amyloid peptide, complexes, copper(II).** ***Environ Health Perspect*** **110(suppl 5):869–870 (2002).** ***http://ehpnet1.niehs.nih.gov/docs/2002/suppl-5/869-870kowalik-jankowska/abstract.html***

Alzheimer disease (AD) is principally a disease of the elderly, although there are small numbers of familial cases. With age, a greater percentage of the population develops the disease, so that by their mid-80s, some 50% of people show signs of AD (*1*). AD is manifested by a series of clinical features. One feature is an impairment of memory, with recent and immediate recall being affected more than remote recall. Another is the loss of ability to perform previously learned complex tasks. Equally symptomatic is the loss of ability to reason.

At the tissue level, the disease is characterized by three typical abnormalities. One is a profound loss of nerve cells inside the brain. The second pathological feature of the disease is microscopic. Fibrous proteins accumulate inside the nerve cells within the cortex of the brain, forming dense mats called neurofibrillary tangles. The neurofibrillary tangles are composed of a phosphorylated form of tau protein (a cytoskeletal protein, i.e., a protein that normally forms the structure within a cell) (*2*). The third feature is currently central to research of senile plaques. This is an aggregation of fibrous proteins in the extracellular space, the area outside a cell and between other cells. Principal among the fibrous proteins in the amyloid plaques is a small protein composed of approximately 39–43 amino acids, known as the β-amyloid (Aβ) peptide (*3*). The Aβ peptide is derived from the larger amyloid precursor protein (APP), a 563–770-residue membrane protein, as a normal cleavage product (*4*).

APP metabolism and processing have been extensively studied, with rats and mice as the most widely used laboratory animal species. However, their Aβ region contains three amino acid substitutions (Arg5 → Gly, Tyr10 → Phe, His13 → Arg) compared with human Aβ peptide (*5*). These changes have been shown to alter the structure and properties of the Aβ peptides (*6*) as well as processing of APP. These sequence alternations are most likely responsible for the virtual absence of Aβ deposits in normal or aged rodent brain (*7*). The importance of Aβ sequence conservation is further strengthened by the occurrence of Aβ deposits in the brains of aged primates, polar bears, and dogs, all known to possess sequence identity to human Aβ peptide (*8*).

Copper and AD

There is substantial interest in the role of copper, manganese, irons, and other redox-active transition metals in the neuropathology of neurodegenerative disorders such as Parkinson disease, AD, and amyotrophic lateral sclerosis. These metals are essential in most biological reactions. However, their excessive tissue accumulation can be cytotoxic, in particular because perturbations in metal homeostasis result in an array of cellular disturbances characterized by oxidative stress and increased free radical production (*9*).

The ability of copper to cycle between stable oxidized Cu^{2+} and unstable reduced Cu^{+} states is used by cuproenzymes involved in redox reactions (e.g., Cu/Zn superoxide dismutase and cytochrome oxidase). However, the $Cu^{2+} \leftrightarrow Cu^{+}$ transitions can in certain circumstances also result in the generation of reactive oxygen species (e.g., superoxide radical and hydroxyl radical), which, if not detoxified efficiently, can damage susceptible cellular components. Copper can also bind with high affinity to histidine and cysteine residues of proteins, which can result in their inactivation. The need to provide copper without the ensuing cellular toxicity has necessitated evolution of tightly regulated copper homeostatic mechanisms (*10*). The brain contains the second highest cellular concentration of copper in the body, next to the liver. Copper is most concentrated in the gray matter (60–110 μM), which is consistently higher than in white matter (25–79 μM) (*11,12*).

The homeostasis of zinc, copper, and iron, and their respective binding proteins is significantly altered in the AD brain (*12,13*). Increased concentrations of copper, iron, and zinc are detected in the neuropil of the AD-affected brain, where they are highly concentrated within amyloid plaques and reach concentrations of up to 0.4 mM for Cu and 1 mM for Fe and Zn (*12*). The observation that Cu/Zn-selective chelators enhance solubility of Aβ peptide from postmortem brain tissue of AD patients and transgenic mouse brains (*14*) suggests that these metal ions may play a role in cerebral amyloid assembly. Copper is probably not an initiator of AD but interacting with APP or its fragments may contribute to the disease development.

APP can reduce Cu^{2+} to Cu^{+} in a cell-free system, potentially leading to increased oxidative stress in neurons (*15*). This copper ion–mediated redox reaction led to disulfide bond formation in APP, which indicated that free sulfhydryl groups of APP are involved. Five years after the initial identification of the amino acid residues involved in the redox reaction, Ruiz et al. confirmed that cysteine 144 is a key residue in the reduction of Cu^{2+} to Cu^{+} by soluble APP (*15,16*). The studies have also shown that copper binding to APP protein in cysteine-rich regions requires the presence of histidine residues. Treatment of neuronal cultures with a peptide corresponding to the human APP copper-binding domain (APP142–166) potentiated copper but not Fe or Zn toxicity. Incubation of APP142–166 with low-density lipoprotein (LDL) and copper resulted in significantly increased lipid peroxidation compared with copper and LDL alone.

Potentially, APP–Cu^{+} complexes may be involved in reducing hydrogen peroxide to form an APP–Cu^{2+}–hydroxyl radical intermediate (*17*). Analysis of the specific reaction revealed the generation of C-terminal polypeptides containing the Aβ domain. The results

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suggest that a cytotoxic gain of function of APP–Cu^+ complexes in the redox reaction with H_2O_2 might result in a perturbation of free radical homeostasis and/or lead to an accumulation of oxidized protein that is known to be associated with AD (*18*).

The Aβ peptide is a normal soluble component of the plasma and the cerebrospinal fluid (*19*). The observation of amyloid deposits in senile plaques in essentially all cases of AD led to the hypothesis that conversion of soluble Aβ into insoluble fibrils is critical for the onset of the disease (*20*). This hypothesis is supported by the fact that fresh Aβ peptide is nontoxic to cultured neurons, whereas aged Aβ (incubated to form amyloid fibrils) becomes toxic (*21*). The studies clearly demonstrate elevated copper levels within neuropil of the cortical and accessory basal nuclei of the amygdala compared with control neuropil, and a further significant elevation of plaque deposits within the core and periphery (*12*).

The results support the interactions of Cu(II) with Aβ because treatment with a copper chelator markedly and rapidly inhibits Aβ accumulation in AD transgenic mice (*22*). Cu(II) induces Aβ aggregation under mildly acidic conditions (e.g., pH 6.8–7.0, such as that believed to occur in the AD brain) (*23*). Rat Aβ1–40 and histidine-modified human Aβ1–40 were not aggregated by Cu^{2+}, indicating that histidine residues are essential for metal-mediated Aβ assembly and perhaps explaining why these animals do not form cerebral Aβ (*23*). Through the use of synthetic peptides corresponding to the first 28 residues of human Aβ, rat Aβ, and single-residue variations, it was revealed that the substitution of Arg for His13 is responsible for the different Zn^{2+}-induced aggregation behaviors of rat and human Aβ. The coordination of Zn^{2+} to His13 is critical to the Zn ion–induced aggregation of Aβ (*24*).

There are three histydyl residues in the Aβ molecule (His6, His13, His14). Raman spectroscopy studies revealed that all three His residues of Aβ are involved in the interactions with Cu^{2+} ions (*25*). Analysis of the Raman spectra has revealed two different modes of metal–Aβ binding. One is characterized by metal binding to the imidazole N_τ of histidine, which produces insoluble aggregates; the other involves metal binding to the N_π atom of histidine as well as to main-chain amide nitrogens, resulting in the formation of soluble complexes. This transition from one binding mode to the other explains the strong pH dependence of Cu(II)-induced Aβ aggregation.

The affinity of copper for Aβ1–42 is seven orders of magnitude higher than for Aβ1–40 (*26*), most likely due to a higher β-sheet content of Aβ42, because this conformation frequently mediates high-affinity copper binding of cuproproteins (*27*). Human Aβ directly produces hydrogen peroxide (H_2O_2) by a mechanism that involves the reduction of Cu(II), setting up conditions for Fenton—type chemistry (*28*). The metal-reducing activity and H_2O_2 production of Aβ species follow the order $A\beta42_{human} > A\beta40_{human} > A\beta40_{mouse} \approx 0$, corresponding to the neurotoxicity and the participation of the native peptides in amyloid pathology (*28*).

The studies on the binding abilities of the various fragments of human and mouse Aβ peptide with Cu^{2+} (fragments 1–6, 1–9, 1–10, 11–16) (*29,30*) have shown that tyrosine residue in the 10th position of the human fragment does not take part in the coordination of the metal ion. The presence of the bulky arginine residue in the 5th position of the peptide sequence of the human fragments changes the coordination ability of the peptide. The presence of histidine residue in the 13th position of human fragment changes the coordination mode of Cu(II) ions compared with the mouse fragment. The human fragment of Aβ peptide is much more effective in Cu(II) ion binding than the mouse fragment because of the presence of the His–His sequence (*30*). These differences in the coordination modes of Cu(II) ions may influence Cu(II) ion–catalyzed oxidation of these peptides by hydrogen peroxide (*31*). The involvement of free radicals in the pathogenesis of AD is now widely accepted for many reasons (e.g., Aβ is sensitive to the action of free radicals) (*32*).

Further studies of Cu(II) complexes with longer fragments (1–16, 1–28, 11–28) of human and mouse Aβ peptide are currently in progress in our laboratory and will be reported soon.

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