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CONTEMPORARY REVIEW

Environmental and Dietary Exposure to Copper and Its Cellular Mechanisms Linking to Alzheimer's Disease

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ABSTRACT

Metals are commonly found in the environment, household, and workplaces in various forms, and a significant segment of the population is routinely exposed to the trace amount of metals from variety of sources. Exposure to metals, such as aluminum, lead, iron, and copper, from environment has long been debated as a potential environmental risk factor for Alzheimer's disease (AD) for decades, yet results from *in vitro*, *in vivo*, and human population remain controversial. In the case of copper, the neurotoxic mechanism of action was classically viewed as its strong affinity to amyloid-beta (Aβ) to help its aggregation and increase oxidative stress via Fenton reaction. Thus, it has been thought that accumulation of copper mediates neurotoxicity, and removing it from the brain prevents or reverse Aβ plaque burden. Recent evidence, however, suggests dyshomeostasis of copper and its valency in the body, instead of the accumulation and interaction with Aβ, are major determinants of its beneficial effects as an essential metal or its neurotoxic counterpart. This notion is also supported by the fact that genetic loss-of-function mutations on copper transporters lead to severe neurological symptoms. Along with its altered distribution, recent studies have also proposed novel mechanisms of copper neurotoxicity mediated by nonneuronal cell lineages in the brain, such as capillary endothelial cells, leading to development of AD neuropathology. This review covers recent findings of multifactorial toxic mechanisms of copper and discusses the risk of environmental exposure as a potential factor in accounting for the variability of AD incidence.

Key words: inflammation; immunotoxicology; neurotoxicity; metals; neurotoxicology, environmental toxicology.

ALZHEIMER'S DISEASE AND RISK FACTORS

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with behavioral and psychological symptoms of dementia (BPSD) among elderly. It is characterized by pathological buildup of amyloid-beta (Aβ) plaques, neurofibrillary tangles (NFTs), and neuroinflammation, together with extensive synaptic and neuronal loss. The gradual but irreversible progression of such neuroanatomical changes in AD takes place over a few decades, yet clinical symptoms, like cognitive decline and BPSD, are not evident until neuropathological features are advanced. To date, aging is known as an essential concomitant of AD, and genetic predisposition also plays a significant role in the onset of this disorder (Bettens *et al.*, 2013). Recent genome-wide association studies and linkage analyses have identified multiple susceptible genes that moderately to substantially increase the risk for late-onset AD (Mukherjee *et al.*, 2017). Heritability for AD is supported by a large-scale twin study indicating that approximately 60% of AD cases have a heritable component (Gatz *et al.*, 1997). However, only 20% of these cases are fully explained by currently known susceptible genes or mutations (Sullivan *et al.*, 2012). Therefore, nonheritable, nongenetic factors are predicted to contribute considerably to triggering neurodegeneration and cognitive decline by interacting with susceptible genes and aging (Sato and Morishita, 2013). In an effort of searching such factors, a number of epidemiological studies and meta-analyses have highlighted potentially modifiable risk factors related to lifestyle, medical history, and

exposure to occupational and environmental pollutants, such as pesticides, polluted air, and heavy metals (Yegambaram *et al.*, 2015). However, the identification of specific environmental risk factors for AD is extremely challenging because of the long latency of the prodromal and cognitively normal phase prior to the clinical manifestations of AD, and because exposure to environmental contaminants could be variable throughout life.

HISTORICAL OVERVIEW OF EXPOSURE TO METALS AND AD—LESSONS FROM ALUMINUM NEUROTOXICITY

Determining a causative relationship between environmental risk factors and the onset of AD has been the subject of criticisms and controversial discussions. Unlike genetic risk factors that are incorporated in individuals throughout their life, environmental risk factors vary depending on their lifestyle, residence, and occupation, making it difficult to tie any particular exposure with the increased onset of AD. In addition, some criticisms argue the lack of convincing evidence on the presence of contaminants in AD brains, epidemiological or population-based studies to associate the exposure with the incident of AD, and neurotoxic mechanisms of contaminants in laboratory models at environmentally relevant dosages. Although fully addressing these criticisms was challenging in earlier works, today's advancement of analytical techniques, methodology, and high-throughput analyses allows us to revisit previously questioned modifiable risk factors once again to discover new cellular and molecular mechanisms of action leading to AD and accelerated cognitive decline. Aluminum (Al) is historically argued on the topic of environmental impact in AD. Epidemiological studies were unable to determine causative link between Al and AD, though a meta-analysis of 9 independent studies where urinary Al concentrations were measured, reported that cognitive performance was impaired with escalating Al concentrations (Meyer-Baron *et al.*, 2007). Advancement of analytical methods using freshly prepared and/or unfixed brain tissues by laser microprobe mass analysis (Bouras *et al.*, 1997), neutron activation (Andrasi *et al.*, 2005), energy-dispersive X-ray spectroscopy with transmission electron microscopy (Yumoto *et al.*, 2009), and luminogallion fluorescent microscopy (Mirza *et al.*, 2017) have later reconfirmed the presence of Al in plaques and NFTs in AD brains. There is, however, the possibility that high Al in AD brains may be an epiphenomenon, not causative but consequent to disruption of the blood brain barrier (BBB) despite the evidence that Al crosses the BBB and disturbs cellular mechanisms to lead impaired cognition in experimental animal models. More comprehensive findings and combined efforts of clinical, epidemiological, and laboratory-based studies will be required to show the causative role of Al, a nonessential metal. Thus, it is not hard to imagine that unveiling a causative link, if any, between copper and AD is even more challenging as it is an essential metal and has critical physiological functions for cellular survival. The major question is how and when the essential metal ever exhibits neurotoxicity.

BENEFICIAL AND DETRIMENTAL ROLE OF COPPER LINKING TO AD

Sources of Environmental Cu Exposure and Its Valency

Copper (Cu) is an essential transition metal serving as a catalytic cofactor for more than 20 enzymes, particularly those involved in cellular respiration and energy metabolism, neurotransmitter biosynthesis, iron metabolism, gene transcription, and antioxidant defense (Itoh *et al.*, 2009). The major source of daily Cu intake is dietary, with approximately 75% from solid food and 25% from drinking water (Brewer, 2015). The preponderance of Cu found in solid food is present in organic molecules as the cuprous Cu^{+} form (Ceko *et al.*, 2014), while that found in drinking water is in the inorganic cupric Cu^{2+} form. This difference in the valency of Cu could result in differential mechanisms of absorption and distribution in the body. The average Cu intake from drinking water is 0.1 to 1 mg/d. To a lesser extent, exposure to polluted air is another source of inorganic Cu. It is released to the atmosphere through anthropogenic processes, such as combustion of fossil fuels and agricultural chemicals, and is associated with particulate matter (PM), dusts, and mists (Fang *et al.*, 2011; Fernandez *et al.*, 2002). Cu in PM is found to be elemental form, a form of oxide, sulfate or carbonate, mostly as its inorganic form of Cu^{2+} (Fernandez *et al.*, 2002). Its concentration in atmosphere ranges from a few nanograms per m^3 to 200 ng/m^3 in the United States, and the concentration may reach up to 5000 ng/m^3 near smelters and mines. When an average individual inhales 11 000 L of air per day, 55 μg of Cu would be inhaled together, potentially associated with long-term environmental and occupational exposure to cause adverse health effects. The impact of Cu exposure through inhalation might elicit greater toxicity in the central nervous system (CNS) than that through gastrointestinal tracts because Cu may acquire the ability to bypass the BBB defense via olfactory nerves when it is associated with fine or ultrafine PM (Block and Calderon-Garciduenas, 2009), as has been shown for manganese on fine PM (Elder *et al.*, 2006; Guarneros *et al.*, 2013).

Cu Absorption, Distribution, and Homeostasis

Brain contains as much Cu as liver does, and its proper distribution, transport, and removal are crucial in order to fulfill its biochemical and physiological functions reactions (Lech and Sadlik, 2007). Brain also have additional network of proteins that bind to Cu and control Cu homeostasis including amyloid precursor protein (APP) and prion protein. These proteins are considered as primary culprits for major neurodegenerative diseases, AD, and Prion disease, respectively.

In the body, Cu is absorbed from the surface of the intestinal microvilli as cuprous (Cu^{+}) by the Cu transporter 1 (CTR1) (Prohaska, 2008), while less is known about the absorption of cupric (Cu^{2+}), which is possibly absorbed through divalent metal transporter 1 or other shared metal transporters. Evolutionally, it appears that essential Cu is primarily supplied from food sources as Cu^{+}. Cu^{2+}, on the other hand, may have limited nutritional value and exhibit dichotomous health effects in the body. A fraction of Cu^{2+} may bypass the normal uptake process and directly enter the blood stream and increase free Cu levels as described below (Medeiros, 2011).

Once in the cell, various Cu chaperones distribute it to organelles and enzymes or excrete it from the cell via specialized Cu transporters, ATP7A and ATP7B. ATP7A is primarily expressed in the basal plasma membrane of intestinal cells to mediate Cu^{+} efflux. ATP7B is highly expressed in the liver and moderately in the brain, and is localized in the transGolgi network of these cells to mediate cellular Cu distribution and excretion. Genetic loss-of-function mutations of ATP7A result in systemic Cu deficiency due to inability of intestinal cells to transport and distribute absorbed Cu^{+} to the rest of the body, and are well-known cause of Menkes disease, while genetic loss-of-function mutations of ATP7B result in excessive

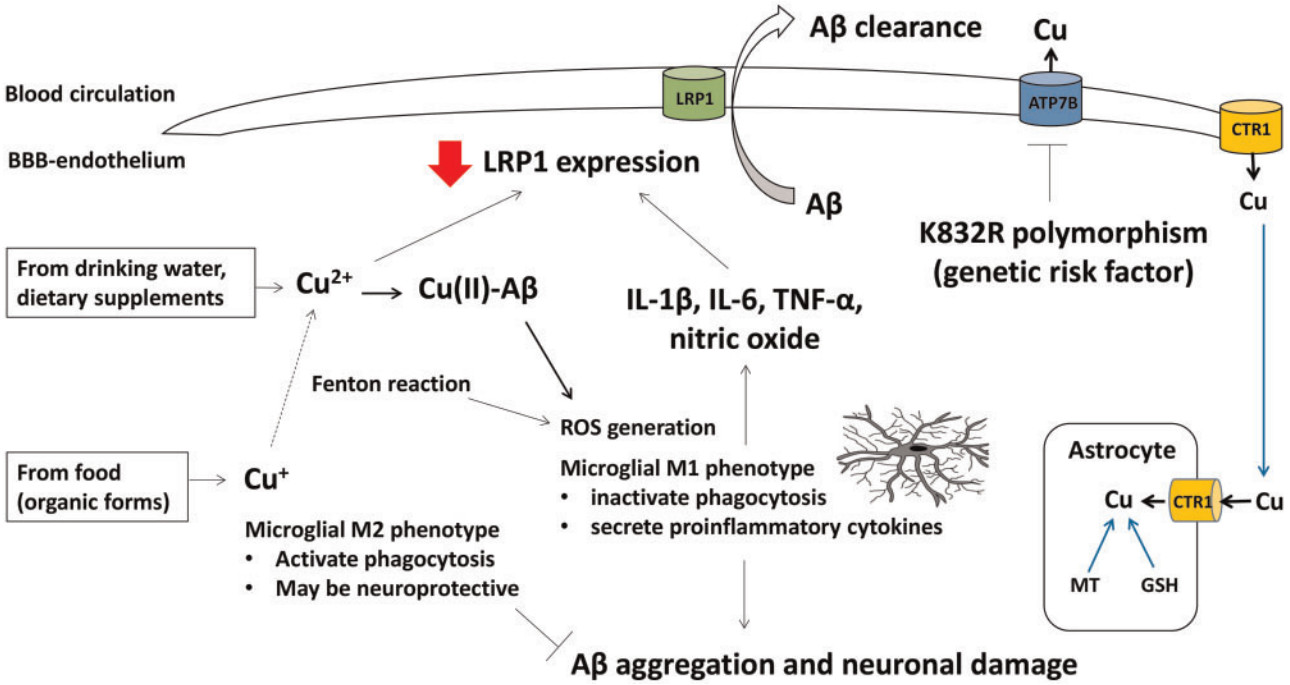

Figure 1. Schematic diagram illustrating the proposed mechanisms of Cu's effects in the brain. BBB is important to modulate Cu transport and Aβ clearance. Cu(I) presents M2 phenotype and prevents inflammation while converted to Cu(II) promotes the down-regulation of LRP1 in capillary endothelial cells, formation of Cu(II)-Aβ complex, ROS generation, and impairment of microglial phagocytosis. In addition, increased proinflammatory cytokine production also down-regulates LRP1, further exacerbating Aβ depositions in brain parenchyma. Astrocytes uptake excess Cu via CTR1 and sequestering it with glutathione (GSH) and metallothionein (MT) to protect cells against reactive oxygen and nitrogen species (ROS/RNS). K832R polymorphism on ATP7B is recently identified as loss-of-function polymorphism, enhances the accumulation of Cu in various organs including brain, and increases the risk for developing AD.

accumulation of Cu particularly in the liver and brain due to its inability to excrete Cu, causing Wilson disease. Both inherited diseases commonly exhibit neurological and neuropsychiatric impairments including cognitive decline, suggesting that both Cu deficiency and excess, in other words, Cu dyshomeostasis in either direction, could trigger severe neurological damage in the brain. Recent genetic evidence to further support the link between Cu dyshomeostasis and AD is the identification of polymorphisms in the ATP7B gene as an increasing risk for AD (Bucossi *et al.*, 2012; Liu *et al.*, 2013), and polymorphic ATP7B is suspected to functional alterations and perturbation of Cu homeostasis (Squitti *et al.*, 2013). Expression of AD-susceptible ATP7B (K832R) polymorphism is determined as the loss-of-function variant in a *Drosophila* model (Mercer *et al.*, 2017), and the presence of ATP7B (K832R) increases the accumulation of Cu in multiple organs including liver and brain, and may exert multiple toxic mechanisms leading to buildup of Aβ species (Figure 1) .

Is Cu a Potential Biomarker to Predict AD?

Most of the Cu in the body is bound to Cu-binding chaperones and enzymes, such as ceruloplasmin (Cp), superoxide dismutase (SOD), catalase, and Cu chaperone for SOD (CCS). In the brain, additional Cu binding proteins, such as APP and prion protein, will bind to Cu and control its homeostasis as described earlier. The amount of dietary Cu intake, however, does not appear to reflect the levels of Cu measured in the serum, CSF, or brain tissues. In AD, CSF Cu levels are inconsistent ranging from elevated to no change or even decreased when compared with nondemented individuals. It is, however, worth noting that a study analyzing 260 subjects found that Cu in CSF is the only metal showing high variability among AD patients (9–109 μg/l, median 18 μg/l) compared with control (13–35 μg/l, median 18 μg/l) (Gerhardsson *et al.*, 2008), suggesting that there is a greater dysregulation of Cu homeostasis in AD brain. Cu levels in the brain tissues from AD is significantly reduced by 13.8% compared with nondemented group by a meta-analysis of 7 available reports (Schrag *et al.*, 2011), and this finding is further supported by recent studies as described later in this review (Akatsu *et al.*, 2012; Xu *et al.*, 2017). On the other hand, the levels of Cu in serum, particularly existing as nonCp-bound Cu, are found to be significantly increased and positively correlated with the severity of cognitive decline, reduced CSF Aβ, and increased CSF tau in mild cognitive impairment (MCI) and AD patients (Squitti *et al.*, 2006, 2011; Talwar *et al.*, 2017; Vural *et al.*, 2010). The exact sources of nonCp-bound Cu in serum remain unknown, though impairment of proper Cu transport and distribution by chaperone proteins or increased exposure to Cu^{2+} and subsequent leakage to the blood stream may be potential factors to elevate these "free" Cu in serum. Individuals with high serum nonCp-bound Cu also convert from MCI to AD by 2.5 years faster than those with low serum nonCp-bound Cu (Squitti *et al.*, 2014), indicating that free Cu in serum could serve as a potential biomarker to predict the progression from MCI to AD. This inverse relationship between Cu and one's cognitive performance has been reported not only among MCI/AD patients but also among cognitively normal individuals (Salustri *et al.*, 2010). However, the environmental origins of Cu exposure or differential metabolic processes causing excess elevated free Cu in serum in these cohorts remain undetermined. Discordance of Cu levels in CSF, brain tissues, and serum in AD patients points to a systemic imbalance of Cu homeostasis and impaired distribution of Cu causing deficiency in the CSF and excess free Cu in the circulation. In this regard, therapeutic

interventions to restore brain Cu levels may promote neuroprotection through upregulating brain's bioenergetics and antioxidative defense (Quinn *et al.*, 2009). However, Cu supplementation may not be a suitable approach to restore brain Cu, and 12-month clinical trial of 8 mg Cu did not rescue nor worsen cognitive outcomes in mild AD patients (Kessler *et al.*, 2008).

On the other hand, among cognitively normal elderly, unintentional overdosing of Cu intake, mainly from over-the-counter nutritional and dietary supplements, together with high dietary fat exhibited almost 5 times accelerated cognitive decline relative to those who had high dietary fat but low daily Cu intake (Morris *et al.*, 2006). Detrimental effect of Cu is potentiated by other dietary factors with unknown mechanisms. High Cu intake by itself did not improve cognitive score, in agreement with finding from the Cu supplementation clinical trial in AD described earlier. Taken together, these findings cannot rule out the possibility that excess intake of inorganic Cu increases the risk of incurring cognitive decline.

Studies to Determine the Link Between Cu and AD

Toxic mechanisms of action of Cu—promoting Aβ buildup and oxidative damage. The physical interaction and binding affinity of Cu to extracellular domain of APP and Aβ species have been extensively studied and shown to promote Aβ production and fibrillization *in vitro* (Atwood *et al.*, 2000; Huang *et al.*, 1999). Within the Aβ sequence, the redox-active Cu^{2+}, but not Cu^{+}, is coordinated to the histidine (His6, His13, and His14) or Tyr10 residues. This coordination leads to formation of the Cu(II)-Aβ complex (predominantly with 1:1 stoichiometry) and aggregation process that involves a conformational change (Tougu *et al.*, 2011; Yugay *et al.*, 2016). Classically, the Cu(II)-Aβ complex is thought to generate reactive oxygen species (ROS) and mediate oxidative damage through Fenton-type reaction and impairment of mitochondrial function (Jiang *et al.*, 2007). This hypothesis further reinforces Cu^{2+} from environmental sources could elicit rather detrimental effects while Cu^{+} from dietary sources fulfills biologically essential demand.

One of early milestone *in vivo* studies investigating Cu exposure in AD was conducted by Sparks and colleagues who reported that chronic exposure to a trace amount of Cu (0.12 ppm), but not zinc or Al, in drinking water, together with high cholesterol (2%) diet for 8 weeks promoted Aβ plaque formation and learning deficits in rodent models (Sparks *et al.*, 2006; Sparks and Schreurs, 2003). Upregulation of ATP-binding cassette transporter A1 (ABCA1) was strongly induced in several brain regions of cholesterol-fed animals with Cu in drinking water but not to the extent with Al or zinc in drinking water (Schreurs and Sparks, 2016). ABCA1 upregulation generally promotes cellular cholesterol efflux through ApoE lipidation and lowers cellular Aβ generation (Kim *et al.*, 2015). High cholesterol diet with Cu in drinking water, however, may outweigh ABCA1-mediated Aβ clearance in the brain of those animals. A recent study reveals that cholesterol-enriched diet significantly increases the levels of N-truncated Aβ species (Perez-Garmendia *et al.*, 2014), which are now commonly detected in the brains from AD and Down syndrome patients within the cores of Aβ plaques (Wirths *et al.*, 2017). N-truncated Aβ species have stronger affinity for Cu^{2+} than naïve forms and promote aggregation of Aβ *in vitro* (Mital *et al.*, 2015), suggesting that the combined effect of Cu and cholesterol leads to accelerated aggregation propensity of Aβ and forms the seeding cores for plaques. Quantification of various N-truncated Aβ species in Cu/cholesterol-fed animals would provide additional key evidence to validate whether this mechanism plays a dominant role in the accelerated buildup of Aβ plaques.

On the contrary, *in vivo* evidence also supports Cu supplementation as protective strategy to reduce Aβ plaques in transgenic mouse model of AD. APP is additional Cu-binding protein in the brain, and mice overexpressing APP transgene develop a reduced brain Cu possibly because excess APP binds to Cu and excrete it from the brain (Bayer *et al.*, 2003; Maynard *et al.*, 2002; Needham *et al.*, 2014). The interaction between APP expression and cellular Cu levels is also demonstrated in cell culture models (Armendariz *et al.*, 2004; Bellingham *et al.*, 2004a,b). Thus, restoration of brain Cu levels in the APP transgenic mouse model by Cu supplementation resulted in the reduction of Aβ production and increased antioxidant capability by stabilizing SOD activity possibly through increased CCS-mediated cellular Cu transport (Bayer *et al.*, 2003). Whether this beneficial effect of Cu observed in mice can be applied directly to humans and AD remains ongoing discussion. To date, although there is no evidence supporting a strong correlation between APP expression and brain Cu levels in AD, Down syndrome, or individuals with APP duplication gene, recent studies carefully quantified Cu in brain tissues by inductively coupled-plasma mass spectrometry and determined a significant reduction of Cu in several brain regions including hippocampus, amygdala, entorhinal cortex, and cerebellum among AD patients (Akatsu *et al.*, 2012; Xu *et al.*, 2017). The reduced levels of cellular Cu in AD brain tissues are comparable of those observed in Menkes disease. Unfortunately, changes in APP levels in these cohorts were not available, so it cannot be determined whether reduction of Cu is mediated by upregulation of APP and buildup of Aβ in the brain, or merely a consequence of extensive neurodegeneration in these regions. In 3xTg-AD mouse model, however, the high dose Cu in drinking water induced detrimental effects include accelerated cognitive impairment and increased Aβ and tau buildup by upregulating the activities of α- and β-secretase and by activating cdk5/p25 (Kitazawa *et al.*, 2009; Yu *et al.*, 2015b). In addition to nonphysiological dose of Cu supplementation, additional PS1 and tau transgenes in 3xTg-AD mice, background strain, duration of the study, and other differences may contribute to the discrepancy of results, but these findings represent complexity of beneficial and toxic effects and mechanisms of Cu, in part may be attributed by its valency, in the biological system.

Metal chelation as a possible therapeutic approach for AD. Initial findings from clinical studies supported that excess Cu in the body contributed to the accelerated Aβ aggregation and buildup, leading to the onset of AD. Thus, removing excess Cu could be a potential therapeutic intervention to ameliorate AD neuropathology. Preclinical testing of metal chelators, clioquinol and DP-109, demonstrated a significant reduction of cerebral Aβ deposit in Tg2576 mice (Cherny *et al.*, 2001; Lee *et al.*, 2004). Shortly after, derivative of clioquinol, PBT2 was introduced in preclinical studies and phase II clinical trials. Despite a remarkable reduction of Aβ and restoration of synapses in animal models, outcomes from clinical trials in prodromal or early stages of AD patients were not promising (Lannfelt *et al.*, 2008). Potential concerns of the use of metal chelators point out that Cu depletion by the chelator may reduce Cu-Aβ interaction and fibrillization, but leading to the buildup of soluble, smaller, and presumably more neurotoxic Aβ species like Aβ dimers in the brain (Goch and Bal, 2017). This is consistent with the idea that Aβ plaques are in fact biologically inert and shielded by microglia processes to contain its neurotoxicity (Yuan *et al.*, 2016).

Novel toxic mechanisms of Cu leading to AD—beyond its interaction with Aβ. In the light of recent findings, Cu may exhibit multiple toxic mechanisms in nonneuronal cell lineages in the brain and promote Aβ buildup without directly interacting with it. Chronic exposure to 0.13 ppm Cu in drinking water in APP23 transgenic mouse model revealed elevated levels of nonCp-bound Cu in plasma and subsequent downregulation of low-density lipoprotein receptor-related protein 1 (LRP1) in capillary endothelial cells (Singh *et al.*, 2013). A parallel loss of LRP1 is observed in advanced aging and in AD brains (Kang *et al.*, 2000; Silverberg *et al.*, 2010), and its genetic ablation in endothelial cells has been shown to cause parenchymal Aβ buildup in mice (Storck *et al.*, 2016). Since this mechanism does not require Cu to cross the BBB and enter the CNS, rather peripheral "free" Cu triggering the loss of LRP1 in endothelial cells, it is well in line with the clinical observations that elevated Cu levels in plasma, but not in CSF or brain, correlate well with the progression and cognitive decline in AD as described earlier. On the other hand, as chronic Cu exposure down-regulates LRP1 in capillary endothelial cell and promotes Aβ buildup, excessive Aβ could further impair its own efflux from brain to blood by oxidizing LRP1, leading to increased Aβ deposition in the brain (Jeynes and Provias, 2008). Thus, Cu toxicity could significantly impair brain's Aβ clearance mechanism and promote Aβ buildup.

In addition to its impact on endothelial cells, Cu plays an important role in maintaining the immune homeostasis (Zucconi *et al.*, 2007), and deficiency or excess Cu differentially alters microglial phenotypes. The involvement of microglia and neuroinflammation in AD has recently emerged as a significant factor and unregulated activation of microglia and astrocytes promotes excessive synaptic pruning, impairment of Aβ clearance, and neurotoxicity (Hong *et al.*, 2016; Liddelow *et al.*, 2017; Yuan *et al.*, 2016). A study using murine monocyte BV2 cells reported that Cu^{+} can polarize cells from a proinflammatory M1 phenotype to a protective antiinflammatory M2 phenotype via inhibition of nitric oxide production (Rossi-George *et al.*, 2012). This finding supports the concept that the cuprous Cu^{+} form acts in a physiologically protective. On the other hand, BV2 cells exposed to Cu^{2+} had impaired phagocytosis and increased release of proinflammatory cytokine, such as IL-1β, IL-6, and TNFα, following Aβ stimulation (Kitazawa *et al.*, 2016), suggesting the cupric Cu^{2+} form perturbs innate inflammatory responses. The Cu(II)-Aβ complex also enhanced microglia-mediated neuronal damage by causing increased production of ROS and nitric oxide, while Cu(II) or Aβ alone did not cause these adverse effects (Yu *et al.*, 2015a). In the TgCRND8 AD mice model, activated microglia might play a modulatory role via uptake of extracellular Cu and upregulation of ATP7A expression to sequester intracellular Cu from Golgi to cytoplasmic vesicles. Therefore, limiting free extracellular Cu, available for complexing with Aβ and subsequent phagocytosis of the Cu(II)-Aβ complex, may prevent plaque formation and inflammation (Zheng *et al.*, 2010). Collectively, the valency of Cu is a critical determinant in the regulation of microglial phenotype and differential toxicity in the brain.

Astrocytes can actively restore and resist against Cu-induced toxicity in part because astrocytes have a high capacity to uptake excess Cu via CTR1 and sequestering it in glutathione and metallothionein complex, key endogenous antioxidant molecules to protect cells against ROS (Bulcke and Dringen, 2015; Scheiber and Dringen, 2011). Astrocyte dysfunction by aging or other means, together with environmental Cu exposure may result in the loss of neuroprotective and antioxidative capacity of the brain and increased susceptibility to cognitive decline and AD.

Overall, results from these studies suggest that not only the direct interaction of Cu with APP or Aβ and neurons, but also the effect of Cu on nonneuronal cell lineages significantly contributes to the development and progression of AD neuropathology (Figure 1). Further research should be directed to provide new insights into potential interactive mechanisms between endothelium, microglia, astrocyte and neuron of Cu exposure.

FUTURE DIRECTIONS

Studies from *in vitro* and *in vivo* models have indicated new ways by which Cu potentiates the hallmark neuropathology of AD. This is in addition to their known ability to interact with Aβ and promote oxidative damage, both of which are generally considered as major neurotoxic mechanisms of action. Cu-induced loss of endothelial LRP1, dysregulation of inflammation, and epigenetic modifications are currently being studied, and their key involvements in AD are soon to be uncovered. Since both neuroinflammation and epigenetic modifications are readily initiated by environmental insults, the current direction of the research further substantiates the capacity of environmental risk factors to facilitate AD.

It is extremely challenging to determine the level of contribution from a single environmental risk factor in humans as dose, duration, route, and timing of the exposure vary from individual to individual, and multiple exposures and conditions are commonly involved throughout one's lifetime. Especially, since Cu is an essential metal, its beneficial effects should always be considered from its biological functions. Sources of Cu exposure and its valency found in the body or brain, but not the absolute amount, may be more relevant with accelerated cognitive decline or the onset of AD. The use of transgenic animal models is still one of valuable research tools to decipher the routes by which Cu and other metals can exert their neurotoxic effects, leading to AD neuropathology. Yet, the selection of appropriate model should be carefully considered especially for Cu study as APP overexpression may significantly lower brain's Cu homeostasis and would not be physiologically relevant to AD. A recent APP knock-in mouse model circumvents this potential issue. In addition, application of unbiased omics techniques will provide supporting evidence and may help identify novel pathways associated with metal toxicity leading to neurodegeneration.

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