BRTE Award Review Article

Neurotoxicity of Trace Elements and the Pathogenesis of Senile-Type Dementia

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Abstract

Both the deficiency and the excess of trace elements can severely damage of the central nervous system. In particular, recent studies have suggested the implication of aluminum and zinc in the pathogenesis of senile-type dementia including Alzheimer's disease and vascular dementia. We investigated the neurotoxicity of aluminum on primary cultured cerebrocortical neurons and found several abnormal changes similar to those observed in Alzheimer's disease. Furthermore, we found that zinc caused the death of cultured neurons, and investigated the underlying molecular mechanism. We also found that disruption of Ca homeostasis may underlie the molecular mechanism of neurotoxicity induced by aluminum or zinc. Our results indicate the significance of trace elements in the brain function and suggest their implications in the pathogenesis of senile-type dementia, including Alzheimer's disease and vascular type dementia.

Keywords : Apoptosis, Ca homeostasis, cultured neuron, Alzheimer's disease, vascular dementia

Introduction

The brain is a major target of environmental toxicants. Although it is a small organ (*ca.* 1.5 kg), it possesses high metabolic capacity. A large volume of blood flows into the brain since it requires a substantial supply of O_2 and glucose. Because neurons are nonproliferative, they retain toxic substances for a long time. Adverse effects on the neuronal circuits during the developmental stage could persist even in adulthood. Although the blood-brain barrier protects the brain from toxicants, it is unable to do this completely. As representative environmental toxicants, trace metals have been the focus of the recent research. Various trace elements are present in the central nervous system, some of which are essential and others, non-essential. It is widely known that essential trace elements such as iron (Fe), zinc (Zn), and copper (Cu) play crucial roles in brain functions. A deficiency in or an excess of abovementioned essential elements could impair neuronal functions and cause severe neurological disorders. Excess intake of non-essential trace elements such as mercury (Hg), aluminum (Al), and lead (Pb) is also implicated in neurodegenerative diseases such as senile-type dementia.

Senile-type dementia is a grave disease occurring in aging human ; its prevalence increases with age[1]. Approximately 25% of old person (85 years and above) are affected. It has been estimated that by the year 2025, 3 million people in Japan will be affected by senile-type of dementia. Senile- type dementia is mainly divided into Alzheimer's disease (AD), vascular dementia (VD), and other types (*e.g.* dementia with Lewy bodies). Increasing evidence suggests a relationship between the dyshomeostasis of trace elements and the pathogenesis of senile-type dementia.

We are interested in the association between trace elements and senile-type dementia. Among the various trace elements found in the brain, we focused on 2

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contrasting elements－Al and Zn－and investigated the molecular mechanism underlying their neurotoxicity. Al is non-essential element despite being abundant in the environment. The relationship between Al and AD has been argued for several decades, but it remains controversial[2-4]. Meanwhile, Zn is the second most abundant trace element in the body and is essential for most living beings[5]. Zn deficiency retards the development of the central nervous system and impairs learning and memory. Despite its significance, numerous studies have suggested that Zn is implicated in the pathogenesis of AD[6] and VD[7-9]. To explore the association between Al and AD, we developed an *in vitro* model system of primary cultured neurons and observed the functional and morphological changes in this system by using the Ca^{2+} imaging system and immunohistochemical techniques. We also found that GT1-7 cells (immortalized hypothalamic neurons) are highly vulnerable to Zn and that the disruption of Ca homeostasis may be based on Zn-induced neuronal apoptosis. In this paper, we review the implications of these 2 trace elements in the pathogenesis of senile-dementia based on the findings of our studies.

Aluminum and Alzheimer's disease

Al is the third most abundant element in the earth's crust and it is wildly distributed in the environment. Despite its abundance, Al is a non-essential element. No known biological reaction that requires Al, owing to several of its specific chemical characteristics. On the contrary, Al inhibits more than 200 biologically important reactions[3,4].

With an increase in pH, the solubility of Al^{3+} decreases, and $Al(OH)_3$ precipitates in a neutral solution. Al favors negatively charged, oxygen-donor ligands. Inorganic or organic phosphates, carboxylate, and deprotonated hydroxy groups are strong Al^{3+} binders[10]. Thus, Al^{3+} binds to the phosphate groups of DNA or RNA, and influences DNA topology and gene transcription. Al^{3+} binds to the phosphate groups of nucleoside di- and triphosphates, such as adenosine triphosphate (ATP), and affects energy metabolism. The phosphate groups of cell membranes are also the binding targets of Al. Furthermore, Al influences the functions of protein kinases and phosphatases. The ligand-exchange rate for Al^{3+} is very low compared with those for other elements. Thus, biological processes involving a Ca^{2+} or Mg^{2+} exchange could be inhibited by substitution with Al^{3+}. These characteristics render Al worthless in enzyme-requiring reactions and may explain its long half-life in the body.

Al has been associated with various neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) and Parkinsonism dementia (PD) in the Kii Peninsula and Guam[11] or with dialysis encephalopathy in dialysis patients[12]. In particular, a relationship between Al and the pathogenesis of AD has been suggested.

The pathological hallmarks of AD are the abnormal deposition of neurofibrillary tangles (NFTs) and senile plaques in the brain[13]. The main constituent of NFTs is the phosphorylated tau protein and that of senile plaques is the β amyloid protein (AβP). A 1965 study showed that the intracerebral administration of Al to experimental animals induced the degeneration of neurofibrils and the appearance of tangle-like structures similar to NFTs in the brains of AD patients[14]. In 1973, Crapper *et al.* reported an increased level of Al in the brains of AD patients[15]. In 1976, Alfrey *et al.* found that Al in dialysis solution or in pharmacological compounds plays a causal role in the encephalopathy in hemodialysis patients (dialysis encephalopathy)[16]. A considerable number of epidemiological studies confirmed a positive relationship between Al in drinking water and the pathogenesis of AD[17,18]. These data support the hypothetical idea, termed the "aluminum hypothesis", that Al contributes to some processes in the pathogenesis of AD and acts as a risk factor. However, despite these lines of evidence, controversy remains, and the aluminum hypothesis has been the subject of much debate in the past few decades[19].

To investigate the validity of the aluminum hypothesis, we have developed a system of primary cultured neurons of the rat cerebral cortex or hippocampus. The cultured neurons could extend neuronal processes, form synaptic contacts, and exhibit electrophysiological activities. The cultured neurons can be maintained for more than 1 month, and their synapses mature during the culture period. These features of *in vitro* cultured neurons, which are similar to those of *in vivo* neurons, facilitate the observation of the pharmacological, functional, and morphological changes induced by Al. We have reported that chronic exposure to Al resulted in the accumulation of the tau protein and AβP, and impaired synapse formation[20,21]. These Al-induced pathological changes in the cultured neurons are similar to the pathological changes observed in AD. We also evaluated the effects of the following Al compounds on cultured neurons : a simple salt of Al^{3+} ($AlCl_3$), a relatively stable

hydrophilic complex (aluminum lactate), a stable membrane-permeable complex [Al $(malt)_3$], and a stable lipophilic complex (aluminum acetylacetonate [Al $(acac)_3$]). We found that Al $(malt)_3$ and Al $(acac)_3$ caused the acute death of cultured rat cerebral cortical neurons [22]. We investigated in detail the characteristics of and the molecular mechanism underlying $Al(malt)_3$-induced neuronal apoptosis, and we found that it was not inhibited by agonists or antagonists of neurotransmitters or by channel blockers, but it was blocked by the application of brain-derived neurotrophic factor (BDNF) [23]. Furthermore, $Al(malt)_3$ inhibits the BDNF-induced increase in the intracellular Ca^{2+} levels ($[Ca^{2+}]_i$), but does not affect glutamate-induced $[Ca^{2+}]_i$ changes. It is widely known that the increase in $[Ca^{2+}]_i$ regulates the expression and various neurotrophic functions of BDNF. Therefore, it is possible that the disruption of Ca homeostasis might be the basis of the mechanism of $Al(malt)_3$-induced neurotoxicity.

Another crucial effect of Al is the conformational change in disease-related proteins including AβP[24]. AβP is a small peptide with 39-43 amino acid residues, and it is derived from the proteolytic cleavage of a large precursor protein, namely, amyloid precursor protein (APP). Yankner *et al.* reported that AβP caused the death of cultured rat hippocampal neurons[25]. Genetic studies have revealed that the 21st chromosome of familial Alzheimer's patients possesses a single point mutation in the APP codon[26]. These lines of evidence support the idea that the accumulation of AβP and the consequent neurodegeneration caused by AβP may underlie the pathogenesis of AD[27]. AβP has an intrinsic tendency to polymerize and form insoluble aggregates with β-pleated sheet structures in an aqueous solution. Substantial evidence shows that the aggregation and subsequent conformational changes occurring in AβP enhance its neurotoxicity[28]. Considering that AβP exists in the cerebrospinal fluid (CSF) even during childhood and that AβP concentration is not elevated in the CSF of AD patients as compared with the controls [29], it is highly possible that the factors that promote or inhibit the aggregation of AβP play crucial roles in its neurotoxicity.

Al possesses the property of binding firmly to various amino acid residues such as tyrosine and histidine and to phosphorylated amino acids, and it acts as a protein cross-linker. We developed a system to investigate AβP polymerization and found that Al enhances the AβP polymerization, and forms SDS-stable oligomers *in vitro* [30]. The aggregated AβP is redissolved by the addition of deferoxamine (DFO) —an Al chelator. Marked polymerization is induced by Al compared to other metals, including Zn, Fe, Cu, and Cd[31]. Furthermore, Al-aggregated AβP binds tightly to the surface of cultured neurons and forms fibrillar deposits, while Zn-aggregated AβP is rarely observed on the surface of cultured neurons[22]. These results suggest that Al-aggregated AβP has a strong affinity to membrane surfaces and are scarcely degraded by proteases.

Moreover, the recent findings of Pratico *et al.* have demonstrated that Al-fed mice transfected with the human APP gene exhibited pathological changes similar to those of the AD brain, *e.g.* a marked increase in the amount of both secreted and accumulated AβP[32]. Increased deposition of senile plaques was also observed. A case study regarding accidental Al-exposure that occurred in 1988 in Camelford (Cornwall, U.K.) revealed that short-term exposure to Al could adversely affect humans with neurological symptoms, demonstrating a rare form of cerebral amyloid angiopathy and an increased deposition of Al[33]. Based on our *in vitro* results and the new lines of evidence mentioned above,

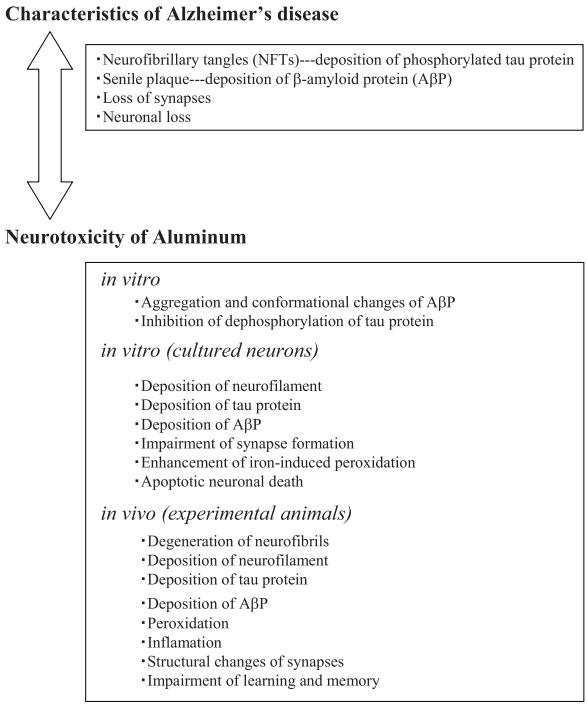

Fig. 1 Comparison between characteristics of Alzheimer's disease and neurotoxicity of aluminum Based on our results and other numerous studies (*see* Ref No. 4 and 19), characteristics of AD and those of Al neurotoxicity are summarized.

we compared the characteristics of AD and Al neurotoxicity as shown in Fig. 1. The similarities between the characteristics of AD and Al neurotoxicity counter the arguments regarding the aluminum hypothesis, making it difficult to refuse[19].

Zinc and Alzheimer's disease

Zn is the second most abundant transition metal in the body. It is essential for most living beings. A considerable amount of Zn accumulates in the brain, particularly in the hippocampus, amygdala, cerebral cortex, and olfactory cortex[34]. The total amount of Zn in the hippocampus is estimated as 70-90 ppm (dry weight). Although in the brain, some Zn is firmly bound to metalloproteins or enzymes, a substantial amount (approximately 10% or more) exists as free zinc ions (Zn^{2+}) or is loosely bound and can be detected by staining with chelating reagents. Chelatable Zn is stored in the presynaptic vesicles of particular excitatory neurons, and during the neuronal excitation, it is secreted from these vesicles to the synaptic clefts with the excitatory neurotransmitter glutamate[35]. Its concentration is estimated to be approximately 300 μM.

It has been reported that Zn alters the behavior of various receptors or ion channels, including the *N*-methyl-D-aspartate (NMDA)-type glutamate, $GABA_A$, glycine, and acetylcholine receptors, ATP channel, voltage-gated Ca^{2+} channel, and K^+ channel. It has been suggested that synaptically released Zn modulates the activity of neuronal circuits in the plasticity the synapses [36]. Accordingly, Zn deficiency during the maternal periods or in the early developmental stages in humans as well as in experimental animals severely damages brain development and impairs learning and memory abilities[37]. Zn deficiency also influences the learning ability and sensitivity of excitatory neurons in adult animals[38].

Increasing evidence has suggested the implication of Zn in the pathogenesis of AD. Bush *et al.* found that Zn remarkably enhances the *in vitro* aggregation of AβP[39]. Zn also binds to APP and modulates the binding of APP to extracellular matrix[40]. APP also binds to Cu and regulates Cu homeostasis[41]. Furthermore, clioquinol (quinoform) －a copper/zinc-sensitive chelator－was reported to inhibit the accumulation of AβP in the brains of experimental animals[42].

However, considering that Zn is abundant in the brain and that even a low concentration (micromolar level) of Zn is sufficient to initiate AβP aggregation, the adverse role of Zn in AD remains disputable. In contrast, the protective role of Zn in the pathogenesis of AD has been suggested. Aripe *et al.* found that AβP forms cation-selective (including Ca^{2+}) ion channels (pores) on artificial lipid membranes[43,44]. We have revealed that AβP forms ion channels on the neuronal cell membranes [45] and causes an abnormal increase in $[Ca^{2+}]_i$[46]. Therefore, it is possible that pore-formation by AβP and the subsequent increase in $[Ca^{2+}]_i$ may trigger apoptotic neurodegeneration and finally induce the pathogenesis of AD[47]. We have demonstrated that Zn inhibits the formation of AβP pores on membranes of neuronal cell membranes[45,48]. Considering that Zn and APP coexist in synapses and are secreted during neuronal excitation, it can be proved that Zn functions as an endogenous blocker of AβP channels. This idea is supported by previous findings which showed that Zn exhibits dual concentration-dependent effects in AβP neurotoxicity : a low concentration of Zn protects neurons, but a high concentration of Zn enhances AβP neurotoxicity[49]. Furthermore, several Zn-related metalloproteins are related to AD. Uchida et al. found that the growth inhibitory factor (GIF), which has the ability of inhibiting the outgrowth of neuronal processes is depleted in AD brain[50]. They revealed that GIF is metallothionein-III (MT-3) which binds to Zn and Cu. Therefore, Zn is undoubtedly implicated in the pathogenesis of AD : however, its role is complex and remains controversial.

Zinc and Vascular type of dementia

Despite its importance, excess Zn can be neurotoxic and is suspected to play a causative role in neuronal injury after transient global ischemia, ultimately leading to the onset of VD. The interruption of blood flow after transient global ischemia induces delayed neuronal death, infarct development, and the subsequent cognitive dysfunction, all of which are believed to be basis of the pathogenesis of VD in elderly people[51]. In response to ischemia, excess glutamate － an excitatory neurotransmitter－is released from the nerve terminals and accumulates in the synaptic clefts. It overstimulates the glutamate receptors and induces the entry of large amounts of Ca^{2+} into the responding neurons. Thereafter, the increased $[Ca^{2+}]_i$ triggers various pathways of apoptotic neuronal death.

Under the ischemic condition Zn is coreleased with glutamate into the synaptic clefts by membrane depolarization. Choi and coworkers have reported that

Zn caused apoptotic death of primary cultured cortical neurons[52-54]. After transient global ischemia, Zn is translocated to vulnerable neurons in the hippocampus prior to the onset of the delayed neuronal death, and the Zn translocation enhances the infarct development. Administration of calcium EDTA (Ca-EDTA) －a Zn-selective membrane-impermeable chelator－inhibited Zn-induced death of cultured cortical neurons, blocked the accumulation of Zn, protected the hippocampal neurons after transient global ischemia, and reduced the infarct volume[55]. These results firmly implicate Zn as a key factor in delayed neuronal death after the transient global ischemia which might be involved in the pathogenesis of VD. However, its detailed mechanism is still under investigation.

We have investigated the mechanism of *in vitro* Zn-induced neurotoxicity by using GT1-7 cells[56,57]. We found that GT1-7 cells are substantially more sensitive to Zn than other neuronal cells including primary cultured neurons of the rat cerebral cortex or hippocampus, PC-12 cells, and B-50 cells[58]. Zn caused apoptotic death of GT1-7 cells in a dose- and time-dependent manner. The GT1-7 cells were developed by Mellon *et al.* by genetically targeting tumorigenesis of mouse hypothalamic neurons[59]. GT1-7 cells possess neuronal characteristics such as extension of neuritis, secretion of gonadotropin-releasing hormone (GnRH), and expression of neuron-specific proteins or receptors including microtubule-associated protein 2 (MAP2), tau protein, neurofilament, synaptophysine, $GABA_A$ receptor, glutamate receptor, dopamine receptor, and L-type Ca^{2+} channels. Meanwhile, the GT1-7 cells lack or possess low levels of ionotropic glutamate receptor and do not exhibit glutamate toxicity[60]. These properties enable us their use as an excellent model system for the investigation of Zn-induced neurotoxicity.

Our pharmacological results demonstrated that the administration of sodium pyruvate, an energy substrate, significantly inhibited Zn-induced death of GT1-7 cells [56]. Therefore, it is possible that energy failure and the inhibition of glycolysis in the mitochondria underlie the mechanism of Zn neurotoxicity. Furthermore, we found that equimolar addition of Al^{3+} and Gd^{3+} markedly inhibited Zn-induced neurotoxicity[61,62]. Moreover, overloading of Ca^{2+} and Mg^{2+} also blocked Zn-induced death of GT1-7 cells; Zn prevented GT1-7 cells from neurotoxicity induced by Ca overload and *vice versa*. These results suggest that Ca dyshomeostasis might be involved in the mechanism of Zn-induced neurotoxicity.

Considering the significance of Zn in ischemia-induced neuronal death, a substance that protects against Zn-induced neuronal death can be a candidate for the prevention or treatment of neurodegeneration after ischemia, and it could ultimately provide a clue regarding the drugs that can be used for VD treatment. We developed a screening system for such substances by using GT1-7 cells, and we found that carnosine (β-alanyl histidine) from fish extracts protects GT1-7 cells against Zn-induced neurotoxicity[63,64]. Carnosine is localized in the neurons of the olfactory bulb and in glial cells, which are not vulnerable to ischemic injuries. Thus, carnosine may be an endogenous protective substance against ischemic neuronal injuries[65]. Figure 2 shows the paradoxical properties of Zn in the pathogenesis of AD and VD.

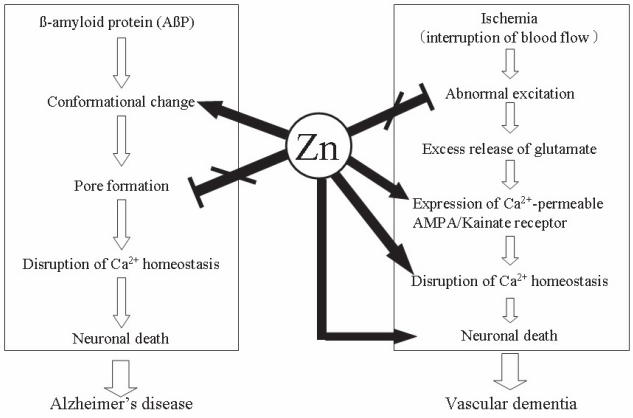

Fig. 2 Paradoxical roles of zinc in the pathogenesis of Alzheimer's disease and vascular dementia. Complex and paradoxical roles of Zn in senile-type dementia are summarized. In the pathogenesis of AD, Zn enhances the conformational changes of AP, while, inhibits the changes of $[Ca^{2+}]_i$ by blocking AP pores. In the pathogenesis of VD, Zn inhibits NMDA-type glutamate channels and regulates the neuronal excitability. However, Zn enhances the ischemia-induced expression of Ca^{2+}-permeable AMPA/Kainate receptor, cause the disruption of Ca^{2+} homeostasis, and neuronal apoptotic death, *etc*.(see details in Ref. 9)

Conclusion

Our *in vitro* study suggested similarities between the properties of Al-induced neurotoxicity and the pathological changes in AD. Even if the association between Al and AD are debatable, it is impossible to deny the neurotoxic properties of Al. Al causes dementia when it accumulates in the brain. Therefore, the

unnecessary intake of Al should be avoided considering its implications on human health.

Our results also indicate the complex and paradoxical roles of Zn in AD and VD. Zn homeostasis may underlie senile-type dementia. In the brain : both the depletion and the excess of Zn can cause severe neuronal damages. In other words, Zn might play a role similar to that of Janus, an ancient Roman god of doorways who has two different faces.

Furthermore, our results suggest Ca dyshomeostasis is implicated in the molecular mechanism of Al-induced neurotoxicity as well as Zn-induced neurotoxicity. The implications of metal-metal interactions in the brain deserve more attention.

In conclusion, our *in vitro* system of cultured neurons provides a useful tool for investigation of the relationship between trace metals such as Al and Zn and senile-type dementia. Further research with regard to these elements will lead to the elucidation of the role of trace elements in the brain and to the development of 'metalloneurochemistry'.

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