— **Review** —

Zinc Neurotoxicity and its Role in Neurodegenerative Diseases

Keiko Konoha, Yutaka Sadakane, and Masahiro Kawahara*

Department of Analytical Chemistry, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714–1 Yoshino-cho, Nobeoka-city, Miyazaki 882–8508, Japan

(Received November 2, 2005)

Zinc is an extremely most abundant trace element in the brain. Substantial amounts of zinc exist in the presynaptic vesicles, and are released with glutamate during the neuronal excitation. Synaptically-released zinc is believed to play crucial roles in normal brain functions. Therefore, zinc deficiency impairs brain development and capabilities of learning and memory. Notwithstanding, recent studies have indicated that excess zinc is linked with several neurodegenerative diseases and has a causative role in delayed neuronal death after transient global ischemia. We have developed the sensitive assay system for zinc neurotoxicity *in vitro* using GT1-7 cells (immortalized hypothalamic neurons) to elucidate the functions of zinc in neurodegenerative diseases. Pharmacological experiments have exhibited the involvement of energy failure, metal-metal interaction, and disruption of calcium homeostasis in zinc-induced neurotoxicity. It is inferred that zinc might play its part in brain functions as Janus, an ancient Roman god with two faces, and that zinc homeostasis is essential for the neuronal survival. Our assay system provides a good method for screening the protective substances of zinc neurotoxicity as a therapeutic target of the global ischemia.

Key words —— calcium homeostasis, vascular dementia, cultured neuron, ischemia, Alzheimer's disease

INTRODUCTION

Zinc is the second most abundant transition metal in the body. It is essential for most living beings. The human body contains approximately 2 g of zinc. It is mainly distributed in the blood, kidney, liver, bone, and brain. Zinc is a co-factor of more than 300 enzymes or metalloproteins. These zinc-related proteins play biologically important roles in mitotic cell division, protein synthesis, DNA and RNA synthesis *etc*. Therefore, zinc is essential for the normal growth and development. In 1961, Prasad *et al*. firstly reported that zinc deficiency caused the dwarfism with the retardation of physical and sexual development in human.[1] Nowadays, zinc deficiency is widely known to impair the overall immunological system, cause adverse effects of the body growth and the sexual development, and lead to olfactory and gustatory dysfunction.[2]

A considerable amount of zinc is accumulated in the brain, particularly in the hippocampus, amygdala, cerebral cortex, and olfactory cortex. The total amount of zinc in the hippocampus is estimated as 70–90 ppm (dry weight).[3] Although some zinc in the brain firmly binds to metalloproteins or enzymes, a substantial amount of zinc (approximately 10% or more) forms free zinc ions (Zn^{2+}) or is loosely bound and detectable by the staining using chelating reagents. Chelatable zinc is stored in the presynaptic vesicles of particular excitatory neurons, and is secreted from vesicles to synaptic clefts with excitatory neurotransmitter glutamate during the neuronal excitation.[4] Its concentration is estimated to be approximately 300 μM.[5]

Despite its abundance, the physiological role of synaptically released zinc has not yet been defined precisely. It has been reported that zinc alters the behavior of various receptors or ion channels, including N-methyl-D-aspartate (NMDA)-type glutamate receptor, γ-aminobutyric acid A ($GABA_A$) receptor, glycine receptor, acetylcholine receptor, ATP channel, voltage-gated Ca^{2+} channel, and K^+

*To whom correspondence should be addressed: Department of Analytical Chemistry, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714–1 Yoshino-cho, Nobeoka-city, Miyazaki 882–8508, Japan. Tel.: +81-982-23-5706; Fax: +81-982-23-5708; E-mail: kawamasa@phoenix.ac.jp

channel.[4] The most established effect of zinc is the modulation of postsynaptic excitability through inhibition of NMDA-type glutamate receptor.[6] A recent zinc-imaging study using newly developed zinc-sensitive fluorescent dye, ZnAF-2, demonstrated that zinc is released activity-dependently from the mossy fiber in the hippocampus, and that zinc modulates activity of neuronal circuits as a spatiotemporal mediator of neuronal signaling and synaptic plasticity.[7] Removal of that synaptic zinc by its chelator induced over-excitation in rat hippocampal neurons.[8] Moreover, synaptically released zinc is required for the induction of long-term potentiation (LTP).[9] These results indicate that zinc is essential for normal brain functions. Accordingly, zinc deficiency during maternal periods or in early developmental stages in human as well as in experimental animals severely damages brain development and impairs learning and memory abilities.[10] Furthermore, zinc deficiency influences learning ability and sensitivity of excitatory neurons in adult animals.[11]

Nonetheless, despite its importance, recent studies have revealed that excess zinc released in a pathological condition is toxic to the central nervous system and the relationship between zinc and neuronal death after traumatic brain ischemia has been specifically examined. Moreover, disruption of zinc homeostasis has been suggested to be implicated in several neurodegenerative diseases including Alzheimer's disease (AD),[12,13] prion disease,[14] amyotrophic lateral sclerosis (ALS),[15] and Wilson's disease.[16] We review here the relationship between zinc neurotoxicity and neurodegenerative diseases and the significance of zinc homeostasis based on our studies and numerous other results.

ZINC AND ISCHEMIA

Interruption of blood flow after transient global ischemia induces delayed neuronal death, the development of an infarct, and subsequent cognitive dysfunction, which are believed to be based on pathogenesis of vascular dementia in elderly people.[17] In response to ischemia, an excitatory neurotransmitter — glutamate — is released from nerve terminals and accumulates in synaptic clefts. Excess glutamate causes over-stimulation of its receptors. Then, it induces the entry of large quantities of Ca^{2+} to responding neurons through NMDA-type glutamate receptors or voltage-dependent Ca^{2+} channels, and thereafter, the increased intracellular Ca^{2+} triggers various pathways of apoptotic neuronal death.[18]

As described above, zinc is co-released with glutamate to synaptic clefts by membrane depolarization in the ischemic condition. Choi and co-workers reported that zinc caused apoptotic death of primary cultured cortical neurons.[19] They also revealed the accumulation of chelatable zinc in degenerating neurons of the hippocampus after transient global ischemia.[20] This zinc translocation occurred in vulnerable neurons in the hippocampus after transient global ischemia but before the onset of the delayed neuronal death, and enhanced the infarct.[21] Administration of calcium EDTA (Ca-EDTA), a zinc-selective membrane-impermeable chelator, inhibited zinc-induced death of cultured cortical neurons,[20] blocked the accumulation of zinc, protected the hippocampal neurons after transient global ischemia,[22] and reduced the infarct volume.[21] These results firmly indicate zinc as a key factor in delayed neuronal death after the transient global ischemia which might be involved in the pathogenesis of vascular dementia.[23] However, its detailed mechanism is still under investigation.

ZINC AND AD

Mounting evidence has suggested the implication of zinc in the pathogenesis of AD. However, it remains still controversial. Although the precise etiology of AD is still not yet clear, it is widely believed that the abnormal deposition of β-amyloid protein (AβP), a major component of senile plaques, in the brain and its neurotoxicity may be based on the molecular mechanism of AD.[24] AβP is a 39–43 amino acid residue peptide derived from a large precursor protein amyloid precursor protein (APP). AβP has an intrinsic tendency to form insoluble aggregates with β-pleated sheet structures. Interestingly, the aggregation and the subsequent conformational change of AβP strongly correlate with its neurotoxicity. Therefore, factors which promote the aggregation of AβP may be involved in the pathogenesis of AD. Bush *et al*. found that zinc remarkably enhance the aggregation of AβP *in vitro*.[25] Zinc also binds to APP and modulates the binding of APP to extracellular matrix.[26] APP also binds to copper[27] and regulates copper homeostasis.[28] Furthermore, clioquinol, a copper/zinc-sensitive chelator, was reported to inhibit the accumulation of AβP in brains of experimental animals.[29]

However, considering that zinc is abundantly

present in the brain and that low concentration (micromolar level) of zinc is enough to initiate the aggregation of AβP, the adverse role of zinc in AD is still disputable. It is possible that zinc influences the homeostasis of other trace metals and contributes to the pathogenesis of AD, because other metals including aluminum, iron, and copper also accelerate the aggregation of AβP.[30] Meanwhile, the protective role of zinc in the pathogenesis of AD has been suggested. Aripe *et al*. found that AβP forms cation-selective (including Ca^{2+}) ion channels on artificial lipid membranes.[31] We have revealed that AβP forms ion channels on neuronal cell membranes[13] and caused the abnormal increase of intracellular calcium level ($[Ca^{2+}]_i$).[32] Therefore, it is possible that channel-formation by AβP and the subsequent increase in $[Ca^{2+}]_i$ may trigger the apoptotic neurodegeneration and finally engenders the pathogenesis of AD.[33] Numerous studies demonstrate that zinc inhibits AβP-channel in lipid bilayer membranes as well as on membranes of neuronal cells.[13,34,35] Considering that both of zinc and APP coexists in synapses and secrets during neuronal excitation, it is provable that zinc may play as an endogenous blocker of AβP channel. It was also reported that zinc has concentration-dependent dual effects in AβP-neurotoxicity: low concentration of zinc protects neurons, but high concentration zinc enhance AβP-neurotoxicity.[36]

Furthermore, there are several zinc-related metalloproteins related with AD. Uchida *et al*. found the protein termed the growth inhibitory factor (GIF) which has the ability of inhibiting the outgrowth of neuronal processes. GIF is abundantly present in the normal brain, however, remarkably depleted in the brain of AD patients. They studied the structure of GIF and found that GIF is metallothionein-III (MT-3) which binds to zinc and copper.[37] Zinc-binding to S100β, a calcium binding protein, influences its binding with hyperphosphorylated tau protein in AD patients.[38] Therefore, there is no doubt about the implication of zinc in the pathogenesis of AD, however, its role is complex and still controversial.

ZINC AND OTHER NEURODEGENERATIVE DISEASE

Prion disease is a transmissible amyloidogenic disease including Creutzfeldt-Jacob disease and Kuru disease in human, and bovine spongiform encephalopathy. The conversion of normal cellular form of prion protein (PrP^C) to the pathogenic protease-resistant form (PrP^{SC}) is believed to be based on the transmission and the pathogenesis of prion disease. Brown *et al*. reported that prion protein has the ability to bind to copper *in vivo*, and that PrP-knockout mice exhibit the reductions in the copper content in the brain.[39] It is hypothesized that PrP^C is a copper-metalloprotein and modulates copper homeostasis. As copper and zinc are competitive in the binding with metalloproteins, zinc also binds to the copper-binding domain of PrP^C. Zinc as well as copper induces the endocytosis of PrP^C,[40] and causes the aggregation of PrP fragment peptide (PrP106-126) and enhances its neurotoxicity.[41] Considering that zinc concentration in the brain is much higher than that of copper, zinc might contribute the functions of PrP^C.

In 1994, linkage analysis of gene of familial ALS patients demonstrated that mutations in the gene of copper, zinc superoxide dismutase (Cu, Zn-SOD) was responsible for the disease.[42] Cu, Zn-SOD is a primary antioxidant enzyme which enables to regulate superoxide. The link between zinc and other neurodegenerative diseases should not be disregarded.

ZINC NEUROTOXICITY *IN VITRO*

We have investigated the mechanism of zinc-induced neurotoxicity *in vitro* to define the role of zinc in ischemic neuronal injury.[43–47] Characterization of zinc neurotoxicity to cultured neuronal cells has been investigated mainly using primary cultured neurons of the rat cerebral cortex[19] or PC-12 cells.[48] However, we found that GT1-7 cells (immortalized hypothalamic neurons) are much more sensitive to zinc than are other neuronal cells.[43,44] The GT1-7 cells were developed by Mellon *et al*. by genetically targeting tumorigenesis of mouse hypothalamic neurons.[49] The cells possess neuronal characteristics such as the extension of neuritis, the secretion of gonadotropin-releasing hormone (GnRH), and the 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. These properties imply that the GT1-7 cell line is a good tool for investigation of neuroendocrine systems[50] or of AD.[32,51] We compared the viability of GT1-7 cells, PC-12 cells, B-50 cells (neuroblastoma cell line), primary cultured

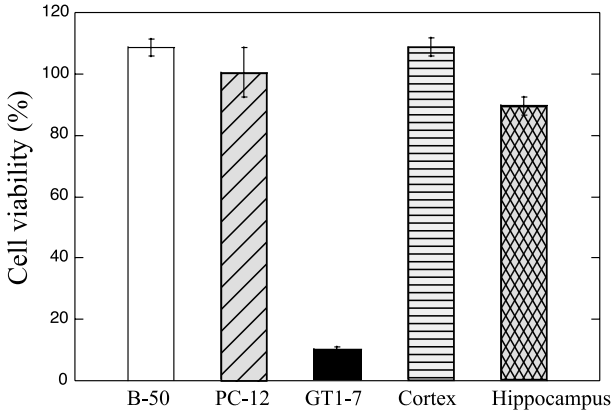

Fig. 1. Zinc-Induced Neurotoxicity on GT1-7 Cells and Other Neuronal Cells

$ZnCl_2$ (50 μM) was administered to B-50 cells, PC-12 cells, GT1-7 cells, primary cultured neurons of rat cerebral cortex (Cortex), or hippocampus (Hippocampus). After 24 hr exposure, viability was measured using WST-1 method. Data are means +/– S.E.M., n = 6. Results are modified from Ref. No. 44.

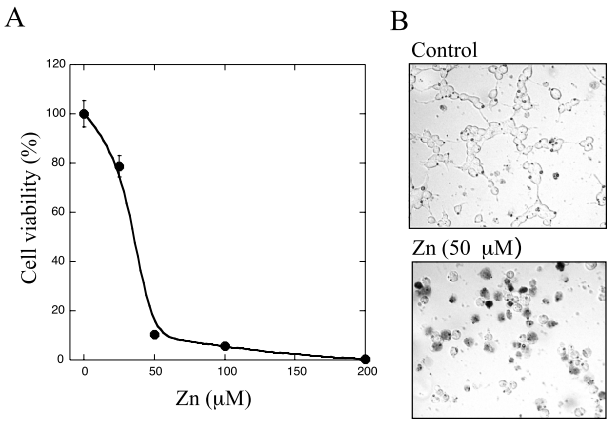

Fig. 2. Zinc Neurotoxicity on GT1-7 Cells

A: Dose–dependency of the viability after zinc exposure. B: Phase contrast images of GT1-7 cells with or without zinc exposure. GT1-7 cells were observed with trypan-blue staining after 24 hr of exposure to $ZnCl_2$ (50 μM).

neurons of rat cerebral cortex, and primary cultured neurons of rat hippocampus after the exposure to zinc (Fig. 1).[44] Among these neuronal cells, GT1-7 cells exhibited the lowest viability after zinc exposure. Zinc caused death of GT1-7 cells in a dose-dependent and time-dependent manner (Fig. 2A).[43] Figure 2B shows phase-contrast images of GT1-7 cells that had been stained with trypan-blue both with and without exposure to zinc. Degenerated GT1-7 cells after zinc exposure exhibit retraction of neuritic processes and cell body shrinkage. These degenerated cells were also terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL) positive and exhibited apoptotic properties.[43] Therefore, we have used the GT1-7 cell line as an excellent model system for investigation of zinc neurotoxicity

MECHANISM OF ZINC-INDUCED NEUROTOXICITY

To elucidate the molecular pathways involved in zinc-induced death of GT1-7 cells, we preadministered various pharmacological compounds and observed changes in viability after zinc exposure. Among tested compounds including agonists or antagonists of neurotransmitters, channel blockers, *etc*., we found that the administration of sodium pyruvate significantly inhibited zinc-induced death of GT1-7 cells.[43] Shelline *et al*. reported that zinc inhibited glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and that pyruvate, an energy substrate, attenuated zinc-induced death of cultured cortical neurons.[52] Therefore, it is possible that the energy failure and the inhibition of glycolysis are based on the mechanism of zinc neurotoxicity also in GT1-7 cells. However, agonists or antagonists of excitatory neurotransimitters [2-amino-5-phosphonovaleric acid (D-APV), glutamate, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)], or those of inhibitory neurotransmitters (bicuculline, muscimol, baclofen, GABA) did not attenuate the viability of GT1-7 cells after zinc exposure.[43–47] These findings are inconsistent with previous findings about the involvement of NMDA type glutamate receptor[19] or alpha-amino-3-hydroxy-5-methyl-4-isoxazole propinic acid (AMPA) type glutamate receptor[53,54] in zinc neurotoxicity in cultured cortical neurons. However, Mahesh demonstrated that the GT1-7 cells lack or possess low levels of ionotropic glutamate receptor and did not exhibit glutamate toxicity.[55] These evidences confirmed that the distinct mechanism of zinc neurotoxicity, which is not mediated by glutamate receptor, might be dominant in GT1-7 cells.

ZINC NEUROTOXICITY AND METAL-METAL INTERACTION

Functions of trace metals are known to be influenced greatly by other metals because many metal-binding proteins share the ability of binding other

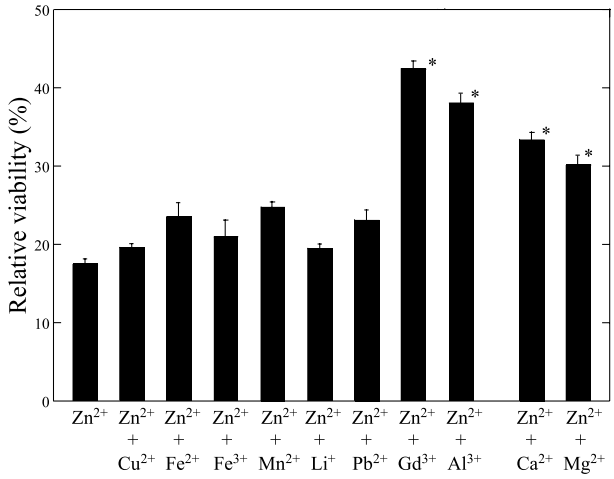

Fig. 3. Effects of Various Metals on Zinc Neurotoxicity to GT1-7 Cells

Various metal solutions including 50 μM of $CuCl_2$ (Cu^{2+}), $FeCl_2$ (Fe^{2+}), $FeCl_3$ (Fe_{3+}), $MnCl_2$ (Mn^{2+}), $LiCl_3$ (Li^{+}), $PbCl_2$ (Pb^{2+}), $GdCl_3$ (Gd^{3+}), $AlCl_3$ (Al^{3+}), or 2 mM of $CaCl_2$ (Ca^{2+}), $MgCl_2$ (Mg^{2+}) were preadministered to GT1-7 cells prior to the exposure to $ZnCl_2$ (50 μM). After 24 hr, the viability was measured using the WST-1 method. For the compensation of endogenous toxicity of the metal, the difference was calculated between the viability of the metal alone and viability of zinc and metal, and described as the relative viability. Data are means +/– S.E.M., $n = 6$. *$p < 0.01$.

metals to a greater or less degree. For example, zinc influences copper homeostasis in Wilson's disease.[16] Iron supplementation influences zinc absorption and *vice versa*.[56] We observed the viability of GT1-7 cells with or without various metal ions after exposure to zinc (Fig. 3),[46,47] and found that equimolar of Al^{3+} and Gd^{3+} significantly inhibited zinc-induced neurotoxicity. Meanwhile, overloading of Ca^{2+} and Mg^{2+} also blocked zinc-induced death of GT1-7 cells.[47] Kim *et al*. reported that zinc neurotoxicity in PC-12 cells was blocked by L-type Ca^{2+} channel blocker.[48] Administration of L-type Ca^{2+} channel blocker attenuated zinc-induced death of GT1-7 cells (data not shown here). Therefore, it is highly possible that dyshomeostasis of calcium might be involved in the mechanism of zinc neurotoxicity.

SCREENING FOR PROTECTIVE SUBSTANCES OF ZINC NEUROTOXICITY

The implication of zinc in transient global ischemia suggests that substances that inhibit zinc neurotoxicity might be candidates for drugs for prevention or treatment of brain ischemia. As noted, zinc chelators were effective in the protection of hippocampal neurons after global ischemia.[20,22] Lee *et al*. reported that intracerebral administration of pyruvate blocked the zinc accumulation as well as the degeneration of hippocampal neurons after ischemia.[57] The GT1-7 cells, which are highly vulnerable to zinc, provide a sensitive assay system for screening such substances. We examined inhibitory effects of various agricultural products on zinc-induced death of GT1-7 cells, and found that the water-soluble extract of mango, *Mangifera indica* L., a subtropical fruit, significantly blocked zinc neurotoxicity.[58] Inhibition was not related with anti-oxidant activity of mango extract. Although we are now determining the structure of the responsive substance, it may become the seed of a new drug for ischemia.

CONCLUSION

Considering these evidences, we have made a hypothetical scheme for zinc neurotoxicity (Fig. 4). In the normal condition, zinc regulates the postsynaptic excitability by the binding to NMDA type glutamate receptor and attenuates AβP neurotoxicity by blocking AβP channel. Altogether, zinc plays protective role for the overexcitation or AβP neurotoxicity. However, in pathological conditions such as ischemia, excess zinc is released in the synaptic cleft with glutamate. Zinc is translocated through the AMPA-dependent Ca^{2+} channel[54] or through other pathways into the target neuron, where zinc inhibits various enzymes, influences mitochondria respiration,[59] and causes energy depletion[52] in the neuron. Excess glutamate induces elevation of intracellular Ca^{2+} level of the target neuron. Zinc also causes the increase in intracellular Ca^{2+} levels and enhances effects of glutamate. The elevated levels of intracellular Ca^{2+} trigger various apoptotic pathways such as the activation of calpain, the activation of caspases or other enzymatic pathways related to apoptosis, and finally engenders to neuronal death.

Mounting evidence indicate that zinc depletion and excess zinc cause severe damage in neurons and learning disorders and that the disruption of zinc homeostasis might engender to several neurodegenerative disorders. However, the role of zinc is still undefined. Zinc might play a role like that of Janus, an ancient Roman god of doorways with two different faces, in the brain. Our developed system using GT1-7 cells will provide a useful tool to define its role in the brain. Further research about the role of

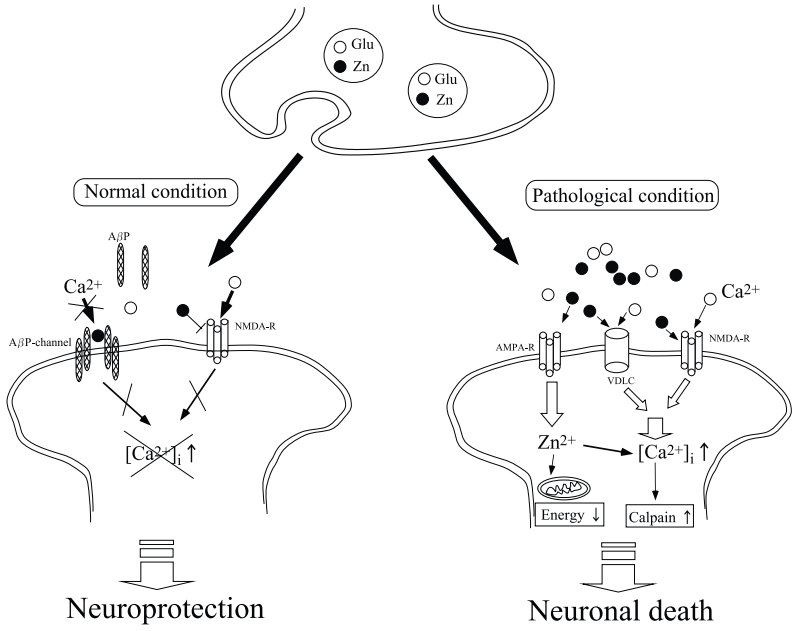

Fig. 4. Hypothetical Scheme of Zinc Neurotoxicity

Zinc (Zn) coexists with glutamate (Glu) in presynaptic vesicles and is secreted with neuronal excitation. In normal conditions, secreted zinc binds to NMDA type glutamate receptor (NMDA-R) and modulates postsynaptic excitability. A*β*P, which also secreted in synaptic clefts, aggregates and forms channel on membranes. Zinc binds to A*β*P channel and inhibits Ca^{2+} influx through the channel. Therefore, zinc plays a protective role. However, in the pathological conditions such as ischemia, great amounts of zinc are released in synaptic clefts and translocated into postsynaptic target neurons through AMPA-type glutamate receptor (AMPA-R)-related Ca^{2+} channel or other pathways such as voltage-dependent L-type Ca^{2+} channel (VDLC). That zinc then inhibits numerous enzymes including mitochondria respiratory enzymes and causes energy depletion. Furthermore, zinc increases intracellular Ca^{2+} levels and enhances effects of glutamate. Increased Ca^{2+} triggers various apoptotic pathways including the activation of calpain, *etc*. Dyshomeostasis of zinc and calcium eventually engenders delayed neuronal death after transient global ischemia.

zinc and its toxicity might engender the development of new treatment for neurodegenerative diseases.

REFERENCES

1) Prasad, A. S., Miale, A., Jr., Farid, Z., Sandsted, H. H., Schulert, A. R. and Darby, W. J. (1961) Biochemical studies on dwarfism, hypogonadism, and anemia. *Arch. Intern. Med*., **111**, 407–428.
2) Hambidge, M. (2000) Human zinc deficiency. *J. Nutr*., **130**, 1344S–1349S.
3) Frederickson, C. J. (1989) Neurobiology of zinc and zinc-containig neurons. *Intern. Review Neurobiol*., **31**, 145–238.
4) Frederickson, C. J., Suh, S. W., Silva, D., Frederickson, C. J. and Thompson, R. B. (2000) Importance of zinc in the central nervous system: the zinc-containing neuron. *J. Nutr*., **130**, 1471S–1483S.
5) Frederickson, C. J., Klitenick, M. A., Manton, W. I. and Kirkpatrick, J. B. (1983) Cytoarchitectonic distribution of zinc in the hippocampus of man and the rat. *Brain Res*., **273**, 335–339.
6) Westbrook, G. L. and Mayer, M. L. (1987) Micromolar concentrations of Zn^{2+} antagonize NMDA and GABA responses of hippocampal neurons. *Nature* (London), **328**, 640–643.
7) Ueno, S., Tsukamoto, M., Hirano, T., Kikuchi, K., Yamada, M. K., Nishiyama, N., Nagano, T., Matsuki, N. and Ikegaya, Y. (2002) Mossy fiber Zn^{2+} spillover modulates heterosynaptic N-methyl-D-aspartate receptor activity in hippocampal CA3 circuits. *J. Cell Biol*., **158**, 215–220
8) Blasco-Ibanez, J. M., Poza-Aznar, J., Crespo, C., Marques-Mari, A. I., Gracia-Llanes, F. J. and Martinez-Guijarro, F. J. (2004) Chelation of synaptic zinc induces overexcitation in the hilar mossy cells of the rat hippocampus. *Neurosci. Lett*., **355**, 101–104.
9) Li, Y., Hough, C. J., Frederickson, C. J. and Sarvey, J. M. (2001) Induction of mossy fiber → Ca3 long-term potentiation requires translocation of synaptically released Zn^{2+}. *J. Neurosci*., **21**, 8015–8025.
10) Bhatnagar, S. and Taneja, S. (2001) Zinc and cognitive development. *Br. J. Nutr*., **85**, S139–S145.
11) Takeda, A. (2000) Movement of zinc and its functional significance in the brain. *Brain Res. Brain Res. Rev*., **34**, 137–148.

12) Huang, X., Cuajungco, M. P., Atwood, C. S., Moir, R. D., Tanzi, R. E. and Bush, A. I. (2000) Alzheimer's disease, beta-amyloid protein and zinc. *J. Nutr*., **130**, 1488S–1492S.
13) Kawahara, M., Arispe, N., Kuroda, Y. and Rojas, E. (1997) Alzheimer's disease amyloid beta-protein forms Zn^{2+}-sensitive, cation-selective channels across excised membrane patches from hypothalamic neurons. *Biophys. J*., **73**, 67–75.
14) Watt, N. T. and Hooper, N. M. (2003) The prion protein and neuronal zinc homeostasis. *Trends Biochem. Sci*., **28**, 406–410.
15) Valentine, J. S. and Hart, P. J. (2003) Misfolded CuZnSOD and amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U.S.A*., **100**, 3617–3622.
16) Brewer, G. J. (2000) Recognition, diagnosis, and management of Wilson's disease. *Proc. Soc. Exp. Biol. Med*., **223**, 39–46.
17) Lee, J. M., Grabb, M. C., Zipfel, G. J. and Choi, D. W. (2000) Brain tissue responses to ischemia. *J. Clin. Invest*., **106**, 723–731.
18) Choi, D. W. (2005) Neurodegeneration: cellular defences destroyed. *Nature* (London), **433**, 696–698.
19) Koh, J. Y. and Choi, D. W. (1994) Zinc toxicity of cultured cortical neurons: involvement of N-methyl-D-asparatate receptors. *Neuroscience*, **4**, 1049–1057.
20) Koh, J. Y., Suh, S. W., Gwag, B. J., He, Y. Y., Hsu, C. Y. and Choi, D. W. (1996) The role of zinc in selective neuronal death after transient global cerebral ischemia. *Science*, **272**, 1013–1016.
21) Lee, J. M., Zipfel, G. J., Park, K. H., He, Y. Y., Hsu, C. Y. and Choi, D. W. (2002) Zinc translocation accelerates infarction after mild transient focal ischemia. *Neuroscience*, **115**, 871–878.
22) Calderone, A., Jover, T., Mashiko, T., Noh, K. M., Tanaka, H., Bennett, M. V. and Zukin, R. S. (2004) Late calcium EDTA rescues hippocampal CA1 neurons from global ischemia-induced death. *J. Neurosci*., **24**, 9903–9913.
23) Weiss, J. H., Sensi, S. L. and Koh, J. Y. (2000) Zn(2+): a novel ionic mediator of neural injury in brain disease. *Trends Pharmacol. Sci*., **21**, 395–401.
24) Hardy, J. and Selkoe, D. J. (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*, **297**, 353–356.
25) Bush, A. I., Pettingell, W. H., Multhaup, G. D., Paradis, M., Vonsattel, J. P., Gusella, J. F., Beyreuther, K., Masters, C. L. and Tanzi, R. E. (1994) Rapid induction of Alzheimer Aβ amyloid formation by zinc. *Science*, **265**, 1464–1467.
26) Bush, A. I., Multhaup, G., Moir, R. D., Williamson, T. G., Small, D. H., Rumble, B., Pollwein, P., Beyreuther, K. and Masters, C. L. (1993) A novel zinc(II) binding site modulates the function of the beta A4 amyloid protein precursor of Alzheimer's disease. *J. Biol. Chem*., **268**, 16109–16112.
27) Hesse, L., Beher, D., Masters, C. L. and Multhaup, G. (1994) The beta A4 amyloid precursor protein binding to copper. *FEBS Lett*., **349**, 109–116.
28) White, A. R., Multhaup, G., Maher, F., Bellingham, S., Camakaris, J., Zheng, H., Bush, A. I., Beyreuther, K., Masters, C. L. and Cappai, R. (1999) The Alzheimer's disease amyloid precursor protein modulates copper-induced toxicity and oxidative stress in primary neuronal cultures. *J. Neurosci*., **19**, 9170–9179
29) Cherny, R. A., Atwood, C. S., Xilinas, M. E., Gray, D. N., Jones, W. D., McLean, C. A., Barnham, K. J., Volitakis, I., Fraser, F. W., Kim, Y., Huang, X., Goldstein, L. E., Moir, R. D., Lim, J. T., Beyreuther, K., Zheng, H., Tanzi, R. E., Masters, C. L. and Bush, A. I. (2001) Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron*, **30**, 665–676
30) Kawahara, M. (2003) Aluminum-induced conformational change and the pathogenesis of Alzheimer's disease. *J. Health Sci*., **49**, 341–347.
31) Arispe, N., Rojas, E. and Pollard, H. B. (1993) Alzheimer disease amyloid β protein forms calcium channels in bilayer membranes: Blockade by tromethamine and aluminum. *Proc. Natl. Acad. Sci. U.S.A*., **90**, 567–571.
32) Kawahara, M., Arispe, N., Rojas, E. and Kuroda, Y. (2000) Alzheimer's β-amyloid, human islet amylin and prion protein fragment evoke intracellular free-calcium elevations by a common mechanism in a hypothalamic GnRH neuronal cell-line. *J. Biol. Chem*., **275**, 14077–14083.
33) Kawahara, M. (2004) Disruption of calcium homeostasis in Alzheimer's disease and other conformational disease. *Curr. Alzheimer Res*., **1**, 87–95.
34) Arispe, N., Pollard, H. B. and Rojas, E. (1996) Zn^{2+} interactions with Alzheimer's amyloid β protein calcium channels. *Proc. Natl. Acad. Sci. U.S.A*., **93**, 1710–1715.
35) Lin, H., Zhu, Y. J. and Lal, R. (1999) Amyloid β protein (1-40) forms calcium-permeable, Zn^{2+}-sensitive channel in reconstituted lipid vesicles. *Biochemistry*, **38**, 11189–11196.
36) Moreira, P., Pereira, C., Santos, M. S. and Oliveira, C. (2000) Effect of zinc ions on the cytotoxicity induced by the amyloid beta-peptide. *Antioxid. Redox. Signal*., **2**, 317–325.
37) Uchida, Y., Takio, K., Titani, K., Ihara, Y. and Tomonaga, M. (1991) The growth inhibitory factor that is deficient in the Alzheimer's disease brain is a

68 amino acid metallothionein-like protein. *Neuron*, **7**, 337–347.
38) Yu, W. H. and Fraser, P. E. (2001) S100beta interaction with tau is promoted by zinc and inhibited by hyperphosphorylation in Alzheimer's disease. *J. Neurosci*., **21**, 2240–2246.
39) Brown, D. R., Qin, K., Herms, J. W., Madlung, A., Manson, J., Strome, R., Fraser, P. E., Kruck, T., von Bohlen, A., Schulz-Schaeffer, W., Giese, A., Westaway, D. and Kretzschmar, H. (1997) The cellular prion protein binds copper in vivo. *Nature* (London), **390**, 684–687.
40) Perera, W. S. and Hooper, N. M. (2001) Ablation of the metal ion-induced endocytosis of the prion protein by disease-associated mutation of the octarepeat region. *Curr. Biol*., **11**, 519–523.
41) Jobling, M. F., Huang, X., Stewart, L. R., Barnham, K. J., Curtain, C., Volitakis, I., Perugini, M., White, A. R., Cherny, R. A., Masters, C. L., Barrow, C. J., Collins, S. J., Bush, A. I. and Cappai, R. (2001) Copper and zinc binding modulates the aggregation and neurotoxic properties of the prion peptide PrP106-126. *Biochemistry*, **40**, 8073–8084.
42) Aoki, M., Ogasawara, M., Matsubara, Y., Narisawa, K., Nakamura, S., Itoyama, Y. and Abe, K. (1993) Mild ALS in Japan associated with novel SOD mutation. *Nat. Genet*., **5**, 323–324.
43) Kawahara, M., Kato-Negishi, M. and Kuroda, Y. (2002) Pyruvate blocks zinc-induced neurotoxicity in immortalized hypothalamic neurons. *Cell. Mol. Neurobiol*., **22**, 87–93.
44) Kawahara, M., Kato-Negishi, M., Hosoda, R. and Kuroda, Y. (2002) Characterization of zinc-induced apoptosis of GT1-7 cells. *Biomed. Res. Trace Elements*, **13**, 280–281.
45) Konoha, K. and Kawahara, M. (2004) Zinc-induced apoptotic death of cultured neuronal cells. *J. Kyushu Univ. Health and Welfare*, **5**, 247–251.
46) Konoha, K., Sadakane, Y. and Kawahara, M. (2004) Effects of gadolinium and other metal on the neurotoxicity of immortalized hypothalamic neurons induced by zinc. *Biomed. Res. Trace Elements*, **15**, 275–277.
47) Konoha, K., Sadakane, Y. and Kawahara, M. (2004) Zinc homeostasis and neuronal death. *Trace Nutrients Res*., **21**, 77–83.
48) Kim, A. H., Sheline, C. T., Tian, M., Higashi, T., McMahon, R. J., Cousins, R. J. and Choi, D. W. (2000) L-type Ca(2+) channel-mediated Zn(2+) toxicity and modulation by ZnT-1 in PC12 cells. *Brain Res*., **886**, 99–107.
49) Mellon, P. L., Windle, J. J., Goldsmith, P. C., Padula, C. A., Roberts, J. L. and Weiner, R. I. (1990) Immortalization of hypothalamic GnRH neurons by genetically targeted tumorigenesis. *Neuron*, **5**, 1–10.
50) Wetsel, W. C. (1995) Immortalized hypothalamic luteinizing hormone-releasing hormone (LHRH) neurons: a new tool for dissecting the molecular and cellular basis of LHRH physiology. *Cell. Mol. Neurobiol*., **15**, 43–78.
51) Yamamoto, H., Hiragami, Y., Murayama, M., Ishizuka, K., Kawahara, M. and Takashima, A. (2005) Phosphorylation of tau at serine 416 by Ca^{2+}/calmodulin-dependent protein kinase II in neuronal soma in brain. *J. Neurochem*., **94**, 1438–1447.
52) Sheline, C. T., Behrens, M. M. and Choi, D. W. (2000) Zinc-induced cortical neuronal death: contribution of energy failure attributable to loss of NAD(+) and inhibition of glycolysis. *J. Neurosci*., **20**, 3139–3146.
53) Weiss, J. H., Hartley, D. M., Koh, J. Y. and Choi, D. W. (1993) AMPA receptor activation potentiates zinc neurotoxicity. *Neuron*, **10**, 43–49.
54) Jia, Y., Jeng, J. M., Sensi, S. L. and Weiss, J. H. (2002) Zn^{2+} currents are mediated by calcium-permeable AMPA/kainate channels in cultured murine hippocampal neurones. *J. Physiol*., **15**, 35–48.
55) Mahesh, V. B., Zamorano, P., De Sevilla, L., Lewis, D. and Brann, D. W. (1999) Characterization of ionotropic glutamate receptors in rat hypothalamus, pituitary and immortalized gonadotropin-releasing hormone (GnRH) neurons (GT1-7 cells). *Neuroendocrinology*, **69**, 397–407.
56) Lonnerdal, B. (2000) Dietary factors influencing zinc absorption. *J. Nutr*., **130**, 1378S–1383S.
57) Lee, J. Y., Kim, Y. H. and Koh, J. Y. (2001) Protection by Pyruvate against Transient Forebrain Ischemia in Rats. *J. Neurosci*., **21**, RC171.
58) Sadakane, Y., Konoha, K. and Kawahara, M. (2005) Protective activity of mango (*Mangifera indica* L.) fruit against a zinc-induced neuronal cell death is independent of its antioxidant activity. *Trace Nutrients Res*., **22**, 78–79.
59) Brown, A. M., Kristal, B. S., Effron, M. S., Shestopalov, A. I., Ullucci, P. A., Sheu, K. F., Blass, J. P. and Cooper, A. J. (2000) Zn^{2+} inhibits alpha-ketoglutarate-stimulated mitochondrial respiration and the isolated alpha-ketoglutarate dehydrogenase complex. *J. Biol. Chem*., **275**, 13441–1347.