Effects of gadolinium and other metal on the neurotoxicity of immortalized hypothalamic neurons induced by zinc

Keiko KONOHA, Yutaka SADAKANE and Masahiro KAWAHARA

Department of Analytical Chemistry, Kyushu University of Health and Welfare

Abstract Zinc is an essential trace element and crucial for numerous biological functions. In contrast, excessive zinc in the synapses plays central roles in the neurodegenerative processes following brain injury such as the transient global ischemia. We have previously demonstrated that zinc caused marked death of immortalized hypothalamic neuronal cells (GT1-7 cells) in a dose- and time- dependent manner. To investigate the molecular mechanism of zinc-induced neurotoxicity of GT1-7 cells, we applied various metal to GT1-7 cells prior to exposure to zinc, and observed the morphological and degenerative changes. Among tested, gadolinium (Gd^{3+}), a widely known channel blocker, significantly blocked zinc-induced cell death in a dose-dependent manner. Aluminum (Al^{3+}) also inhibited zinc neurotoxicity. Our results suggest that functions of Gd^{3+} such as the modification of calcium homeostasis are implicated in the mechanism of zinc-induced neurotoxicity.

Key words: zinc, GT1-7, gadolinium, aluminum, apoptosis, ischemia

Introduction

Zinc (Zn) is the second abundant trace element in the body and contributes to various biological functions. Numerous studies indicated that zinc deficiency leads to the impairment of immune system, the delay of body growth, the disorder of smell and taste, and the retardation of cognitive development [1,2]. Zn is concentrated in the central nervous system, and exists as a constituent of metalloproteins or localizes as ionic form in the synaptic vesicles [3]. Vesicular Zn is released to synaptic clefts with neurotransmitters during the neuronal excitation. Zn influences various receptors or ion channels including NMDA-type glutamate receptors, GABA receptors, voltage-gated calcium channels [4], and may modulate the neuronal information [5]. Furthermore, Zn prevents neuronal death in several neurodegenerative disorders such as Alzheimer's disease [6, 7]. In contrast, recent studies have suggested that excessive amount of extracellular Zn plays central roles in neurodegenerative processes following the brain injury such as global ischemia or epilepsy [8]. Koh *et al.* reported that the accumulation of Zn in degenerating neurons after transient forebrain ischemia and the prevention of such neuronal death by a zinc chelating agent [9]. They also reported that Zn caused death of primary cultured rat cerebral cortical neurons, astrocytes, or PC12 cells [10-12]. These evidences support the idea that Zn neurotoxicity is implicated in the mechanism underlying neurodegeneration after the global ischemia. However, the molecular mechanism of Zn-induced neurotoxicity remains elusive.

Corresponding author: Masahiro Kawahara
Department of Analytical Chemistry, 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

Accepted: Sep 13, 2004

We have found that Zn caused marked death of immortalized hypothalamic neurons (GT1-7 cells) [13]. GT1-7 cells were derived from site-specific tumorigenesis of murine hypothalamic neurons and widely used as the model neurons for neuroendocrine studies [14]. Zn caused apoptotic death of GT1-7 cells in the lower concentration compared to primary cultured neurons of rat cerebral cortex or hippocampus [15]. Our previous results have shown that Zn-induced neurotoxicity of GT1-7 cells was not influenced by agonists or antagonists of neurotransmitters (glutamate, D-APV, GABA, bicuculline), or by a channel blocker (tetrodotoxin), but significantly inhibited by the preadministration of metal chelators or sodium pyruvate, an energy substrate. Pyruvate is reported to attenuate Zn-induced toxicity in various cell types including cultured cortical neurons [16] and to block the neurodegeneration *in vivo* induced by global ischemia [17]. Therefore, it is possible that some agents which could attenuate zinc neurotoxicity become candidates for drugs for the treatment of neurodegeneration following transient global ischemia. To search for such preventive substances and to elucidate the molecular mechanism of Zn-induced neurotoxicity, we focused on effects of other metals in this article. Although Zn coexists with other various metals in the brain, little is known about the metal-metal interactions. Therefore we preadministrated several metals to GT1-7 cells with or without Zn and observed changes in the viability and in morphology.

Materials and methods

Cell culture

GT1-7 cells were cultured as described previously [13]. Briefly, the cells were grown in Dulbecco's modified Eagle's medium/Nutrient Mixture F-12 Ham (DMEM/F-12) supplemented with 10% fetal calf serum. Upon reaching confluence, the cells were dissociated by incubation for 5 min at 37C° in trypsin-EDTA solution. After enzymatic dissociation, cells were resuspended in serum-free DMEM

and plated on 96-well culture dishes at a concentration of $3x10^5$ cells/cm^2. The cells were incubated for one day in a humidified atmosphere of 93% air and 7% CO_2 at 37 °C.

Cell viability assay

GT1-7 cells were plated on 96-well culture dishes at a concentration of $3x10^5$ cells/cm^2. After 1 day in vitro, the solutions of $ZnCl_2$ were added to cell culture medium. After 24 h of the exposure, we examined viability of the cells using a commercially available kit (Cell Counting Kit, Dojindo Chemicals). The WST-1 assay used here is a modification of the MTT assay which measures the activity of cellular mitochondorial dehydrogenase [18]. To examine effects of metals, the solutions of various metals were preadministerated to culture media just prior to the exposure to Zn. Metals were obtained from Wako Pure Chemicals Industries Ltd. and dissolved in distilled water.

Morphological observation

GT1-7 cells were plated on polyethylenimine-coated coverslips at a concentration of 1.25 x 10^5 cells/cm^2 and maintained in serum-free DMEM for two days. After 24 hours exposure to the solutions of $ZnCl_2$, the cells were stained by membrane-permeable dye, (DiI: 1, 1-dioctadecyl-3, 3, 3', 3'-tetramethylindocarbocyanine perchlorate), and were observed under a fluorescent microscope (IX71; Olympus).

Results and Discussion

We have previously demonstrated that 25-100 μM $ZnCl_2$ caused marked death of GT1-7 cells in a dose- and time-dependent manner [13]. To examine effects of various metal ions on Zn-induced cell death, we administered various metals (each 50 μM; iron ($FeCl_2$, $FeCl_3$), manganese ($MnCl_2$), copper ($CuCl_2$), lead ($PbCl_2$), lithium (Li_2CO_3), gadolinium ($GdCl_3$), aluminum ($AlCl_3$)) in culture media of GT1-7 cells prior to the exposure to 50 μM of Zn^{2+}. The viability of cells was determined using the WST-1 method (Fig. 1).

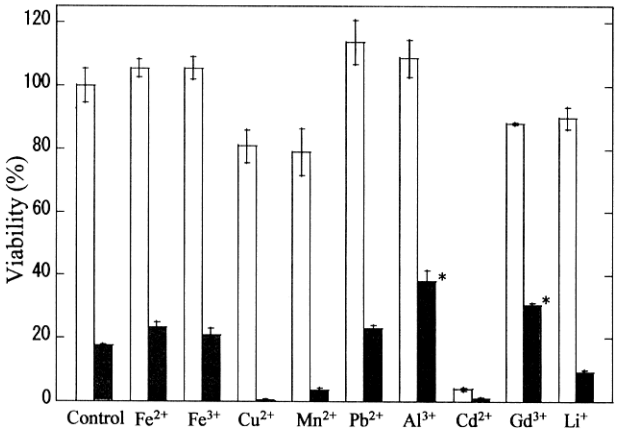

Fig. 1 Effects of various metals on zinc-induced neurotoxicity of GT1-7 cells

Fifty μM of various metal ions including Fe^{2+} ($FeCl_2$), Fe^{3+} ($FeCl_3$), Cu^{2+} ($CuCl_2$), Mn^{2+} ($MnCl_2$), Pb^{2+} ($PbCl_2$), Gd^{3+} ($GdCl_3$), Al^{3+} ($AlCl_3$), Li^+ (Li_2CO_3) were applied to GT1-7 cells with (closed square) or without 50μM of Zn^{2+}(open square). After 24 h, the cell viability was analyzed using WST-1 method. Data are expressed as mean ± SEM, n=6., * significance at $p<0.01$ vs. control with Zn^{2+}.

After 24 h of the exposure to 50 μM $ZnCl_2$, 82.1 ± 5.4% of GT1-7 cells were degenerated (mean ± SEM, n=6). Metal ions such as Cu^{2+}, Mn^{2+} are slightly toxic without Zn^{2+}, and Cd^{2+} caused the degeneration of most GT1-7 cells in this experimental condition. Preadministration of metals including Fe^{2+}, Fe^{3+}, Pb^{2+} and Li^+ did not influence Zn-induced death of GT1-7 cells. However, gadolinium (Gd^{3+}) and aluminum (Al^{3+}) significantly inhibited the effect of Zn, while Gd^{3+} itself did not influence the cell viability. Figure 2 shows that Gd^{3+} attenuated Zn-induced neurotoxicity in a dose-dependent manner. Co-existence of 200 μM of Gd^{3+} with 40 μM Zn^{2+} blocked the toxicity and the cell viability was raised to 91.5 ± 3.3%, while the viability of GT1-7 cells exposed to 40 μM Zn^{2+} was 47.7 ± 0.6% (mean ± SEM, n=6). Figure 3 shows the morphological changes of GT1-7 cells. In this culture condition, GT1-7 cells differentiated and extend neuronal processes (Fig. 3A). However, after 24 hours of Zn exposure (30 μM), GT1-7 cells were degenerated with spherical, swollen cell bodies and retracted processes (Fig. 3B). The preadministration of Gd^{3+} (200 μM) inhibited the morphological degenerative changes caused by Zn (Fig. 3C).

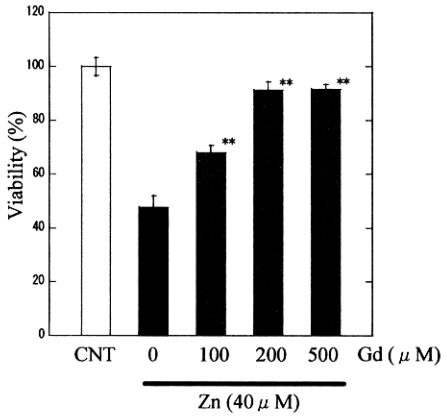

Fig. 2 Dose-dependent effects of gadolinium on zinc-induced neurotoxicity

Various concentrations of Gd^{3+} ($GdCl_3$) were applied to GT1-7 cells prior to the exposure to Zn^{2+} (40 μM). After 24 h, the cell viability was analyzed using WST-1 method. Data are expressed as mean ± SEM, n=6., ** significance at $p<0.001$ vs. Zn^{2+} without Gd^{3+}.

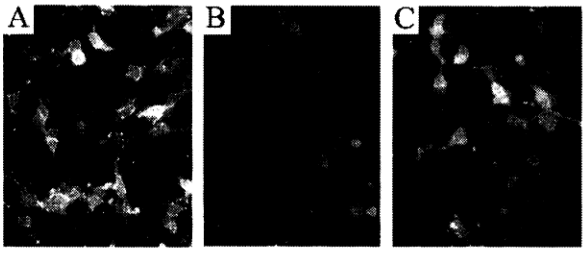

Fig. 3 Morphological changes of GT1-7 cells

After 3 days in vitro, GT1-7 cells were stained by DiI and were observed their morphological changes. A: control, B: GT1-7 cells exposed to 30 μM of Zn^{2+} for 24h, C: GT1-7 cells preadminiterated with 200 μM of Gd^{3+} prior to Zn^{2+}.

Our results demonstrated that several metals such as Gd^{3+} or Al^{3+} inhibited Zn-induced neurotoxicity of GT1-7 cells. Although other ions such as Fe^{2+}, Fe^{3+}, Li^{3+} *etc.* showed no

significant changes, it should be noted that the ionic forms of these metals are labile and easily changed in the culture media which contains various metal-binding chemicals. Therefore, it is difficult to determine whether these metals act in the described ionic form or not. Further studies are necessary for the determination of interactions of metal ions with Zn-induced neurotoxicity more precisely. In spite of the methodological limitations, it is notable that Gd^{3+} attenuated the degeneration of GT1-7 cells caused by Zn in a dose-dependent manner. Although the inhibitory mechanism of Gd^{3+} on Zn-induced neurotoxicity is under investigation, Gd^{3+}, a lanthanide ion, is a widely known channel blocker. Gd^{3+} was reported to inhibit various channels such as voltage-gated Ca^{2+} channels [19] or Ca^{2+}-activated K^+ channels [20], and to influence kainate or AMPA receptor functions [21, 22]. Considering that several studies have suggested the implication of Ca^{2+} channels in Zn-induced toxicity [12], it is plausible that the modification of Ca^{2+} homeostasis by Gd^{3+} play important roles in the preventive mechanism of Zn neurotoxicity. In other respect, it is also possible that Gd^{3+} inhibited Zn influx into GT1-7 cells as Shelline *et al.* previously found in cultured cortical neurons [22]. Further research using Zn^{2+}-imaging technique will be useful for elucidating the precise inhibitory mechanism of Gd^{3+}.

Gd^{3+} is used as a nontoxic agent for magnetic resonance imaging. Therefore, it is probable that Gd^{3+} or related compounds can become a candidate agent for treatment of global ischemia. Our developed model system for investigating Zn neurotoxicity will provide a good tool for search and estimate such substances.

References

1. Hambidge M: Human zinc deficiency. J Nutr 130: 1344S-1349S, 2000.
2. Takeda A, Minami A, Takefuta S, Tochigi M, Oku N: Zinc homeostasis in the brain of adult rats fed zinc-deficient diet. J Neurosci Res 63:447-452, 2001.
3. Frederickson CJ, Suh SW, Silva D, Frederickson CJ, Thompson RB: Importance of zinc in the central nervous system: the zinc-containing neuron. J Nutr 130: 1471S-1483S, 2000.
4. Harrison NL, Gibbons SJ: Zn^{2+}: an endogenous modulator of ligand- and voltage-gated ion channels. Neuropharmacology 33: 935-952, 1994.
5. Ueno S, Tsukamoto M, Hirano T, Kikuchi K, Yamada MK, Nishiyama N, Nagano T, Matsuki N, Ikegaya Y: Mossy fiber Zn^{2+} spillover modulates heterosynaptic N-methyl-D-aspartate receptor activity in hippocampal CA3 circuits. J Cell Biol 158: 215-220, 2002.
6. Kawahara M, Arispe N, Kuroda Y and Rojas E: Alzheimer's disease amyloid ß-protein forms Zn^{2+}-sensitive, cation-selective channels across excised membrane patches from hypothalamic neurons, Biophys J 73: 67-75, 1997.
7. Lovell MA, Xie C, Markesbery WR: Protection against amyloid ß peptide toxicity by zinc. Brain Res 823: 88-95, 1999.
8. Weiss JH, Sensi SL, Koh JY: Zn^{2+}: a novel ionic mediator of neural injury in brain disease. Trends Pharmacol Sci 21: 395-401, 2000.
9. Koh JY, Suh SW, Gwag BJ, He YY, Hsu CY, Choi DW: The role of zinc in selective neuronal death after transient global cerebral ischemia. Science 272:1013-1016, 1996.
10. Koh JY, Choi DW: Zinc toxicity of cultured cortical neurons: involvement of N-methyl-D-asparatate receptors. Neuroscience 4: 1049-1057, 1994.
11. Sheline CT, Takata T, Ying H, Canzoniero LM, Yang A, Yu SP, Choi DW: Potassium attenuates zinc-induced death of cultured cortical astrocytes. Glia 46: 18-27, 2004.
12. Kim AH, Sheline CT, Tian M, Higashi T, McMahon RJ, Cousins RJ, Choi DW: L-type Ca(2+) channel-mediated Zn(2+) toxicity and modulation by ZnT-1 in PC12 cells. Brain Res 886: 99-107, 2000.
13. Kawahara M, Kato-Negishi M, and Kuroda Y: Pyruvate blocks zinc-induced neurotoxicity in immortalized hypothalamic neurons. Cellular and Molecular Neurobiology 22: 87-93, 2002.
14. Mellon PL, Windle JJ, Goldsmith PC, Padula CA, Roberts JL, Weiner RI: Immortalization of hypothalamic GnRH neurons by genetically targeted tumorigenesis. Neuron 5: 1-10 (1990).
15. Kawahara M, Kato-Negishi M, Hosoda R, Kuroda Y: Characterization of zinc-induced apoptosis of GT1-7 cells. Biomed. Res. Trace Elements 13: 280-281, 2002.
16. Sheline CT, Behrens MM, Choi DW: Zinc-induced cortical neuronal death: contribution of energy failure attributable to loss of NAD(+) and inhibition of glycolysis. J Neurosci 20: 3139-3146, 2000.
17. Lee JY, Kim YH, Koh JY: Protection by Pyruvate against transient forebrain ischemia in rats. J Neurosci 21:RC171, 2001.
18. Ishiyama M, Tominaga H, Shiga M, Sasamoto K, Ohkura Y, Ueno K: A combined assay of cell viability and in vitro cytotoxicity with a highly water-soluble tetrazolium salt, neutral red and crystal violet. Biol Pharm Bull 19: 1518-1520, 1996.
19. Mlinar B, Enyeart JJ: Block of current through T-type calcium channels by trivalent metal cations and nickel in neural rat and human cells. J Physiol 469: 639-652, 1993.
20. Imai T, Okamoto T, Yamamoto Y, Tanaka H, Koike K, Shigenobu K, Tanaka Y: Effects of different types of K+ channel modulators on the spontaneous myogenic contraction of guinea-pig urinary bladder smooth muscle. Acta Physiol Scand 173: 323-333, 2001.
21. Huettner JE, Stack E, Wilding TJ: Antagonism of neuronal kainate receptors by lanthanum and gadolinium. Neuropharmacology 37:1239-1247, 1998.
22. Lei S, MacDonald JF: Gadolinium reduces AMPA receptor desensitization and deactivation in hippocampal neurons. J Neurophysiol 86:173-182, 2001.