New Insights into Neurozinc and Metallothioneins

Review

Antimicrotubule Agent-Induced Zinc Neurotoxicity

Bo Young Choi and Sang Won Suh*
Department of Physiology, College of Medicine, Hallym University; Chuncheon 24252, Korea.
Received February 28, 2018

Colchicine or vincristine depolymerize microtubules, an action which blocks neuron axonal transport. Thus, these chemicals showed selective neurotoxicity in hippocampal neurons. However, the mechanism of neurotoxicity by these antimicrotubule agents has remained unclear. Our previous studies have suggested that colchicine-induced hippocampal neuron death is caused by incremental increases in intraneuronal free zinc. We have demonstrated that zinc transporter 3 gene deletion (ZnT3−/−) reduces dentate granule cell death after colchicine injection. This ZnT3−/−-mediated reduction of dentate granule cell death was accompanied by a decrease in the incidence of oxidative injury. Unexpectedly, we found that ZnT3−/− mice contain a higher glutathione (GSH) level in the hippocampal neurons than wild type mice. Thus, ZnT3−/− mice showed less neuronal GSH depletion by colchicine injection, and thus less neuronal death. These results suggest that the higher levels of neuronal GSH in ZnT3−/− mice result in less dentate granule cell death after colchicine injection. In addition to colchicine, our lab also demonstrated that a chemotherapeutic agent, pacritaxel (Taxol), which is a microtubule stabilizing agent, depleted vesicular zinc in the presynaptic terminals and induced a reduction of neurogenesis. Therefore, in the present review, we discussed how antimicrotubule agent-induced neurotoxicity and cognitive impairment is associated with zinc dyshomeostasis in the brain.

Key words zinc; colchicine; taxol; zinc transporter 3; dentate granule cell; neurogenesis

1. INTRODUCTION

Microtubules are important cytoskeletal components in neurons; they provide platforms for axonal transport involved in a variety of cellular functions, including the transport of synthesized proteins and secretory vesicles. Colchicine binds tubulin, one of the major components of microtubules. Since tubulin is involved in the formation of crucial components of cellular mitosis, including neurons, colchicine essentially acts as a “mitotic poison.” Since cancer cells show a higher rate of mitosis, they are more vulnerable to colchicine. However, since colchicine is also toxic to normal cells, the therapeutic value of this chemical against cancer has been limited.

Three decades ago, Goldschmidt and Steward first demonstrated that hippocampal dentate granule cells degenerate after colchicine injection. The intracerebral injection of colchicine induces damage of hippocampal CA1 pyramidal neurons, which results in cognitive impairment. Our previous study has demonstrated that colchicine or vincristine induce hippocampal neuron death by an incremental increase in intracellular free zinc. Therefore, we have suggested a new neuronal death mechanism, induced by blocking axonal transport and subsequent incremental increase in intracellular free zinc as the intermediary step in antimicrotubule agent-induced neuron death.

Chemotherapy-induced cognitive impairment (CICI) has been reported in many patients undergoing cancer therapy. This CICI is often called “chemobrain” as it includes memory impairments, difficulty with word associations, confusion and slowed thinking processes during chemotherapy. Our current understanding of how CICI occurs or how we can prevent it is still not clear. Since many anticancer agents are derivatives of antimicrotubule chemicals, a possible relationship between CICI and zinc-induced neuronal death has been suggested.

Zinc is one of the most important transition metals in our bodily functions—one of the most important elements for DNA synthesis, cell division and many other physiological functions. Most of the zinc in the neuronal cytoplasm comes in a protein-bound form. Free or chelatable zinc is located in the vesicles of synaptic terminals. Axonal transport of zinc between the body and synaptic terminals is essential for neuronal function. Abnormal axonal transport has been shown to occur in several central nervous system (CNS) disorders, such as amyotrophic lateral sclerosis (ALS). Thus, in the present review, we discuss a possible role of zinc in antimicrotubule agent-induced neuronal death and cognitive impairment.

2. COLCHICINE-INDUCED NEURON DEATH IS MEDIATED BY FREE ZINC INCREMENT

The addition of colchicine resulted in the selective loss of hippocampal dentate granule cells. However, the mechanism of the colchicine-induced dentate granule cell death and depletion of mossy fibers is unclear. Cytoplasmic components, including zinc, are transported by axonal transport from the cytoplasm to the nerve terminals. Thus, colchicine may block microtubule-assisted axonal zinc transport between cytoplasm and synaptic terminals. With this hypothesis, our lab recently demonstrated that colchicine induced incremental increase in free zinc in the dentate granule cells.

The mechanism of zinc transport from cytoplasm to the...
synaptic terminal is unknown. If zinc is incorporated in the cytoplasm and then transported to the synaptic terminals, these processes can be altered by antimicrotubule agents, such as colchicine or vincristine. Furthermore, if the incremental increase in free zinc in cytoplasm continues after colchicine injections, then the free zinc level continues to increase until neuronal death. Therefore, our previous studies have demonstrated that colchicine-induced granule cell death is induced by intraneuronal free zinc.\(^6\) This study demonstrated four lines of evidence: (i) colchicine administration induced an incremental increase in intraneuronal free zinc in the hippocampal dentate granule cells; (ii) colchicine induced zinc depletion from the synaptic terminals; (iii) extracellular zinc chelator administration showed no protection against the above phenomena; and (iv) another antimicrotubule agent, vincristine, induced similar patterns. These results support our hypothesis that antimicrotubule agents induce dentate granule cell death by incremental increases in intraneuronal free zinc (Fig. 1).

3. COLCHICINE-INDUCED NEURON DEATH IS REDUCED BY ZnT3 GENE DELETION

Previous studies have demonstrated that colchicine injections caused increased oxidative stress, which resulted in cognitive impairment.\(^{21-23}\) Several lines of evidence have demonstrated that colchicine induces oxidative stress by the following mechanisms: (i) Colchicine increases nitric oxide (NO) production in the brain,\(^{24}\) and the increase in NO can cause the peroxynitrite-mediated formation of free radicals by interaction with the superoxide anion. (ii) The generated NO also induces the nitrosylation of diverse enzymes, thereby inducing neuronal death.\(^{25}\) Therefore, the generation of oxidative stress induced by colchicine injection can cause dentate granule cell death.

Glutathione (GSH) plays an important role in the antioxidant defense against free radical production. An excess of reactive oxygen species (ROS) production causes GSH depletion.\(^{26-28}\) The onset of cell death is related to the depletion of intracellular GSH level.\(^{29,30}\) Therefore, we can attenuate oxidative stress by increasing antioxidant defense mechanisms via the modulation of GSH, which can be an effective strategy to prevent further neuronal death.\(^{31-33}\)

Our previous study has demonstrated that the injection of colchicine caused dentate granule cell death by increased oxidative stress and incremental increases in free zinc.\(^6\) Our lab also demonstrated that this colchicine-induced dentate granule cell death was reduced after zinc transporter 3 (ZnT3) gene deletion.\(^{34}\) ZnT3 is localized at the synaptic vesicles in the brain. Yoo et al.\(^{35}\) demonstrated that the level of free zinc (unbound form of zinc) is higher in the neuronal cytoplasm of ZnT3 gene deletion mice than in wild-type (WT) mice. Metallothioneins 1 and 2 (Mt1/2), which are known to be induced by increases in cytosolic free Zn\(^{2+}\) levels, were also substantially increased in ZnT3 gene deletion mice. Interestingly, several studies have demonstrated that the level of free zinc modulates GSH level in the cells.\(^{36-39}\) Parat et al.\(^{40}\) demonstrated a positive correlation between intracellular zinc reduction and GSH reduction in cells treated by the cell permeable zinc chelator \(N,N,N',N'-\text{tetrakis(2-pyridylmethyl)}\text{-ethylenediamine} (\text{TPEN})\).\(^{40}\) Our previous study also found that ZnT3 gene deletion resulted in a higher GSH level than that in WT mice. We found that ZnT3 gene deletion resulted in less GSH depletion after colchicine injection, thereby inducing a lesser incremental increase in free zinc and thus less dentate granule cell death.\(^{34}\) The mechanism by which ZnT3 gene deletion causes increased intraneuronal zinc levels and increased
neuronal GSH levels remained unclear. However, our published work demonstrated that increased intracellular free zinc levels by ZnT3 gene deletion may cause increases in neuronal GSH level, which has been shown to provide neuroprotective effects after colchicine injection (Fig. 2).

4. CHEMOTHERAPEUTIC AGENT PACLITAXEL REDUCES VESICULAR ZINC LEVELS AND DECREASES HIPPOCAMPAL NEUROGENESIS

Hippocampal neurogenesis plays an important role in maintaining normal brain function. Several studies have demonstrated that elevating the capacity of neurogenesis enhances hippocampal-dependent learning and memory tasks in rodents, while decreasing this capacity impairs such tasks. Neuronogenesis-specific problems have been reported in CICI. Neurogenesis in the sub-granular zone of the hippocampus is reduced by chemotherapeutic agents. Our recent published work has suggested that reduced vesicular zinc is related to the CICI-induced reduction of neurogenesis and accompanying cognitive decline.

Our lab has demonstrated that zinc may be an essential element for hippocampal neurogenesis. Vesicular zinc is most highly localized in the hippocampal mossy fibers of dentate granule cells. We reported that dietary zinc deficiency reduced hippocampal neurogenesis and impairs short-term memory tasks. We also demonstrated that increasing zinc concentrations by dietary supplementation increases hippocampal neurogenesis. Based on the previous studies cited above, we used a chemotherapeutic agent, paclitaxel (Px), to test our hypothesis that zinc may be related to CICI by a reduction of hippocampal neurogenesis. Similar to colchicine, Px is also an antimicrotubule agent and as such also reduces axonal transport. We found that Px treatment reduced the differentiation of progenitor cells into neuroblasts. Px also reduced vesicular zinc levels and ZnT3 expression. These results suggest that reduced vesicular zinc pools in hippocampal synaptic terminals by Px may interfere with neurogenesis and therefore lead to the cognitive decline seen in patients after chemotherapy. Thus, we have proposed that zinc supplements can be used as a simple alternative treatment to ameliorate CICI. However, further clinical investigation in humans is needed.

5. CONCLUSION AND FUTURE DIRECTIONS

In this review, we focused on a potential relationship between zinc and neuron death or between zinc and neurogenesis under the conditions of blocked axonal transport induced by antimicrotubule agents. It became clear that cytoskeleton-disrupting agents, including colchicine or vincristine, cause hippocampal dentate granule cell death by blockade of the axonal zinc transport and subsequent incremental increases in intraneuronal free zinc. The genetic deletion of ZnT3 leads to an increase in neuronal GSH levels, thereby reducing colchicine-induced oxidative injury and dentate granule cell death. Furthermore, Px, a microtubule stabilizing agent, reduces vesicular zinc levels and decreases hippocampal neurogenesis.

However, the molecular mechanisms underlying those findings have not been fully understood. Further studies will be required to add more mechanistic detail to this process.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Vale RD. The molecular motor toolbox for intracellular transport. Cell, 112, 467–480 (2003).
2) Uppuluri S, Knippling L, Sackett DL, Wolff J. Localization of the colchicine-binding site of tubulin. Proc. Natl. Acad. Sci. U.S.A., 90, 11598–11602 (1993).
3) Goldschniedt RB, Steward O. Preferential neurotoxicity of colchicine for granule cells of the dentate gyrus of the adult rat. Proc. Natl. Acad. Sci. U.S.A., 77, 3047–3051 (1980).
4) Emerich DF, Walsh TJ. Cholinergic cell loss and cognitive impairments following intraventricular or intradentate injection of colchicine. Brain Res., 517, 157–167 (1990).
5) Nakagawa Y, Nakamura S, Kase Y, Noguchi T, Ishihara T. Colchicine lesions in the rat hippocampus mimic the alterations of several markers in Alzheimer’s disease. Brain Res., 408, 57–64 (1987).
6) Choi BY, Lee BE, Kim JH, Sohn M, Song HK, Chung TN, Suh SW. Colchicine induced intraneuronal free zinc accumulation and dentate granule cell degeneration. Metallomics, 6, 1513–1520 (2014).
7) Dietrich J, Monje M, Wefel J, Meyers C. Clinical patterns and...
biological correlates of cognitive dysfunction associated with cancer therapy. Oncologist, 13, 1285–1295 (2008).

8) Vardy J, Rourke S, Tannock IF. Evaluation of cognitive function associated with chemotherapy: a review of published studies and recommendations for future research. J. Clin. Oncol., 25, 2455–2463 (2007).

9) Bower JE. Behaviorial symptoms in patients with breast cancer and survivors. J. Clin. Oncol., 26, 768–777 (2008).

10) Vardy J, Tannock I. Cognitive function after chemotherapy in adults with solid tumours. Crit. Rev. Oncol. Hematol., 63, 183–202 (2007).

11) Davis J, Ahlberg FM, Berk M, Ashley DM, Khassraw M. Emerging pharmacotherapy for cancer patients with cognitive dysfunction. BMC Neurol., 13, 153 (2013).

12) Mar Fan HG, Clemons M, Xu W, Chemerynski I, Breunis H, Lasek RJ. Axoplasmic transport of labeled proteins in rat ventral motor neurons. Exp. Brain Res., 21, 1185–1192 (2011).

13) Lee BE, Choi BY, Hong DK, Kim JH, Lee SH, Kho AR, Kim H, Choi HC, Suh SW. The cancer chemotherapeutic agent paclitaxel (Taxol) reduces hippocampal neurogenesis via down-regulation of vesicular zinc. Sci. Rep., 7, 11667 (2017).

14) Cull RE. Role of axonal transport in maintaining central synaptic connections. Exp. Brain Res., 24, 97–101 (1975).

15) Takeda A, Kodama Y, Ohnuma M, Okada S. Zinc transport from the striatum and substantia nigra. Brain Res. Bull., 47, 103–106 (1998).

16) Williamson TL, Cleveland DW. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. Nat. Neurosci., 2, 50–56 (1999).

17) Craddock TJ, Tuszyński JA, Chopra D, Casey N, Goldenste LE, Hameroff SR, Tanzi RE. The zinc dyshomeostasis hypothesis of Alzheimer’s disease. PLOS ONE, 7, e33552 (2012).

18) Goldschmidt RB, Steward O. Neurotoxic effects of colchicine: differential susceptibility of CNS neuronal populations. Neurosci. Lett., 7, 695–714 (1982).

19) Lasek RJ. Axoplasmic transport of labeled proteins in rat ventral motoneurons. Exp. Neurol., 21, 41–51 (1968).

20) Veerendra Kumar MH, Gupta YK. Intracerebroventricular administration of colchicine produces cognitive impairment associated with oxidative stress in rats. Pharmacol. Biochem. Behav., 73, 565–571 (2002).

21) Kumar A, Seghal N, Naidu PS, Padi SS, Goyal R. Colchicine-induced neurotoxicity as an animal model of sporadic dementia of Alzheimer’s type. Pharmacol. Rep., 59, 274–283 (2007).

22) Kumar A, Dogra S, Prakash A. Neuroprotective effects of Centella asiatica against intracerebroventricular colchicine-induced cognitive impairment and oxidative stress. Int. J. Alzheimer’s Dis., 2009, 972178 (2009).

23) DuFournay L, Leroy D, Warembourg M. Differential effects of colchicine on the induction of nitric oxide synthase in neurons containing progesterone receptors of the guinea pig hypothalamus. Brain Res. Bull., 52, 435–443 (2000).

24) Gallus SR, Panizzon KL, Henry D, Wasterlain CG. Neuroprotection against nitric oxide injury with inhibitors of ADP-ribosylation. Neuroreport, 5, 245–248 (1993).

25) Buttte TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. Immunol. Today, 15, 7–10 (1994).

26) Schulz JB, Lindenau J, Seyfried J, Dicgans J. Glutathione, oxidative stress and neurodegeneration. Eur. J. Biochem., 267, 4904–4911 (2000).

27) Dringen R. Metabolism and functions of glutathione in brain. Prog. Neurobiol., 62, 649–671 (2000).

28) Beaver JP, Waring P. A decrease in intracellular glutathione concentration precedes the onset of apoptosis in murine thymocytes. Eur. J. Cell Biol., 68, 47–54 (1995).

29) Bains JS, Shaw CA. Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. Brain Res. Brain Res. Rev., 25, 335–358 (1997).

30) Marksberry WR. Oxidative stress hypothesis in Alzheimer’s disease. Free Radiol. Biol. Med., 23, 134–147 (1997).

31) Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidiant therapeutic options. Curr. Neuropharmacol., 7, 65–74 (2009).

32) Li J, O W, Li, Wang ZG, Ghanbari HA. Oxidative stress and neurodegenerative disorders. Int. J. Mol. Sci., 14, 2438–2447 (2013).

33) Dufourny L, Leroy D, Warembourg M. Differential effects of colchicine on the induction of nitric oxide synthase in neurons containing progesterone receptors of the guinea pig hypothalamus. Brain Res. Bull., 52, 435–443 (2000).
50) Perez-Clausell J. Distribution of terminal fields stained for zinc in the neocortex of the rat. *J. Chem. Neuroanat.*, 11, 99–111 (1996).

51) Suh SW, Won SJ, Hamby AM, Yoo BH, Fan Y, Sheline CT, Tamao H, Takeda A, Liu J. Decreased brain zinc availability reduces hippocampal neurogenesis in mice and rats. *J. Cereb. Blood Flow Metab.*, 29, 1579–1588 (2009).

52) Golub MS, Takeuchi PT, Keen CL, Gershwin ME, Hendrickx AG, Lonnerdal B. Modulation of behavioral performance of prepubertal monkeys by moderate dietary zinc deprivation. *Am. J. Clin. Nutr.*, 60, 238–243 (1994).

53) Choi BY, Kim IY, Kim JH, Lee BE, Lee SH, Kho AR, Sohn M, Suh SW. Zinc plus cyclo-(his-pro) promotes hippocampal neurogenesis in rats. *Neuroscience*, 339, 634–643 (2016).

54) Kampan NC, Madondo MT, McNally OM, Quinn M, Plebanski M. Paclitaxel and its evolving role in the management of ovarian can-

55) Nabholz JM, Tonkin K, Smylie M, Au JJ, Lindsay MA, Mackey J. Chemotherapy of breast cancer: are the taxanes going to change the natural history of breast cancer? *Expert Opin. Pharmacother.*, 1, 187–206 (2000).

56) LaPointe NE, Morfino G, Brady ST, Feinstein SC, Wilson L, Jordan MA. Effects of eribulin, vincristine, paclitaxel and ixabepilone on fast axonal transport and kinesin-1 driven microtubule gliding: implications for chemotherapy-induced peripheral neuropathy. *Neurotoxicology*, 37, 231–239 (2013).

57) Shemesh OA, Spira ME. Paclitaxel induces axonal microtubule polar reconfiguration and impaired organelle transport: implications for the pathogenesis of paclitaxel-induced polyneuropathy. *Acta Neuropathol.*, 119, 235–248 (2010).