Effects of selenium-containing compounds on Cu^{2+}/Zn^{2+}-induced neuronal cell death and potential application as therapeutic agents for neurological diseases

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In many tissues, trace metals such as iron (Fe), zinc (Zn), and copper (Cu) are important for various physiological functions such as immune function, cell division, and enzyme function. However, it has been shown in humans and experimental animal models that excessive amounts of these trace metals in the body can induce various diseases in the central nervous system, liver, and respiratory tract. Although the role of zinc in the central nervous system is controversial, with some reports suggesting a protective role, we are interested in the negative effects of excessive amounts of zinc on the central nervous system. Previous studies suggest that zinc, which is released in excessive amounts after ischemic injury, is a major modulator of neuronal death, and that Zn^{2+}-induced neuronal death is an important cause of dementia after ischemic injury (Koh et al., 1996). In addition, other trace metals are present in the brain and/or cerebrospinal fluid, and during neuronal excitation, Cu^{2+} accumulated in synaptic vesicles is released into the synaptic cleft (Opazo et al., 2014). As the concentrations of these trace metals have been noted to increase, especially under pathological conditions, our research group speculated that these metal-metal interactions may induce neuronal cell death and influence the onset and exacerbation of neurological diseases.

Therefore, we have been analyzing the mechanisms by which zinc and other metals induce neuronal cell death. Recently, using immortalized hypothalamic neuronal cells (GT1-7 cells), we found that non-toxic concentrations of Cu^{2+} exacerbate Zn^{2+}-induced neurotoxicity by producing reactive oxygen species (ROS), endoplasmic reticulum (ER) stress response, and mitochondrial damage (Tanaka and Kawahara, 2017; Tanaka et al., 2018a, b). We also focused on the stress-activated protein kinase/c-Jun amino-terminal kinase (SAPK/JNK) signaling pathway and examined its involvement in Cu^{2+}/Zn^{2+}-induced neurotoxicity. We found that co-treatment with Cu^{2+} and Zn^{2+} increases activated forms of SAPK/JNK (p46 and p54), c-Jun, and activating transcription factor 2 in GT1-7 cells. In addition, we found that oxidative stress is a key modulator of Cu^{2+}/Zn^{2+}-induced activation of the SAPK/JNK signaling pathway (Tanaka et al., 2019).

On the basis of these results, we are searching for compounds that can inhibit Cu^{2+}/Zn^{2+}-induced neuronal cell death with the aim of developing therapeutic agents for neurological diseases. We have found that pyruvate, which is involved in various metabolic pathways such as the glycolytic pathway and the tricarboxylic acid cycle, suppresses Cu^{2+}/Zn^{2+}-induced neuronal cell death through its inhibitory effect on cytochrome c release from mitochondria (Tanaka et al., 2018a). We also found that SP600125, a SAPK/JNK inhibitor, suppressed Cu^{2+}/Zn^{2+}-dependent activation of the SAPK/JNK signaling pathway and neuronal cell death (Tanaka et al., 2019). Furthermore, we found that HSA-Trx, a derivative of the antioxidant thioredoxin, inhibits Cu^{2+}/Zn^{2+}-induced mitochondrial damage, ER stress response, and SAPK/JNK pathway activation via inhibition of ROS production (Tanaka et al., 2018b, 2019). Taken together, these results suggest that oxidative stress is located upstream of Cu^{2+}/Zn^{2+}-dependent mitochondrial damage, ER stress, and SAPK/JNK pathway activation. Therefore, we believe that compounds with antioxidant properties, such as HSA-Trx, are promising therapeutic agents for the treatment of neurological diseases in which Cu^{2+}/Zn^{2+}-induced neuronal cell death is involved in the pathogenesis and exacerbation.

Selenium (Se) is an essential trace element for the synthesis of antioxidant enzymes, but the difference between the required amount and the toxic amount is smaller than that of other trace elements. Excessive intake of selenium can cause gastrointestinal disorders, peripheral neuropathy, and skin disorders, but because selenium-dependent enzymes play an important role in the body, humans and animals must consume selenium in the form of seleno-L-methionine and seleno-L-cysteine. Glutathione peroxidase (GPX) and thioredoxin reductase are typical selenium-dependent enzymes that function as antioxidant enzymes to remove lipid peroxide and hydrogen peroxide from the body. The protective effects of selenium-containing compounds against neurological diseases have been reported using cellular and animal models. Sereno-L-methionine has been reported to reduce glial activation, alleviate neuroinflammation, and attenuate neuronal cell death in the olfactory bulb of a mouse model of Alzheimer's disease (AD) (Zhang et al., 2016). It has been reported that selenite pretreatment improves cerebral infarct volume and decreases DNA oxidation, that selenium inhibits glutamate-induced cell death and oxidative stress in hippocampal HT22 neuron cells, and that sodium selenite protects against 3-nitropropionic acid-induced oxidative stress by increasing GPX activity in cultured primary cortical neurons (Mehta et al., 2012; Ma et al., 2017). These results suggest that selenium-containing compounds may have a protective effect against neurological diseases through their antioxidant effects.

However, because the effect of selenium-containing compounds on Cu^{2+}/Zn^{2+}-induced neuronal cell death has not been tested, we recently analyzed the effect of seleno-L-methionine on Cu^{2+}/Zn^{2+}-induced neuronal cell death. To summarize the results, seleno-L-methionine markedly inhibited Cu^{2+}/Zn^{2+}-induced neuronal cell death, ER stress response, and activation of the SAPK/JNK signaling pathway in GT1-7 cells. Furthermore, seleno-L-methionine markedly inhibited the production of ROS, an upstream factor in Cu^{2+}/Zn^{2+}-dependent neuronal cell death, through the induction of the antioxidant protein GPX1. Thus, we believe that seleno-L-methionine has the potential to become a therapeutic agent that can inhibit the onset and exacerbation of neurological diseases involving neuronal cell death (Nakano et al., 2020; **Figure 1**).

However, there are still some problems that must be solved if seleno-L-methionine is to be clinically applied for the treatment of neurological diseases. As described above, the difference between the effective dose and the toxic dose of selenium is small, so the determination of the clinical dose of selenium-containing compounds must be done carefully. Although the side effects of selenium-containing compounds in humans have been described above, several adverse events caused by selenium-containing compounds have also been reported in animal and cellular models. It has been reported that treatment of Hela cells with seleno-DL-cysteine induces two morphologically distinct types of cell death, one with an apoptosis-like phenotype and the other with a paraptosis-like phenotype (Wallenberg et al., 2014). It has also been reported that selenium induces ROS and

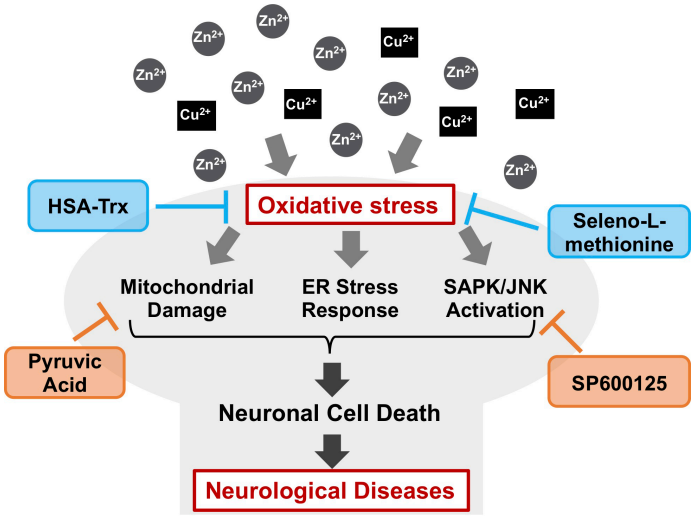

Figure 1 | **Mechanisms of Cu^{2+}/Zn^{2+}-induced neuronal cell death and compounds that inhibit neuronal cell death.**
Cu: Copper; ER: endoplasmic reticulum; HSA-Trx: human serum albumin-thioredoxin 1 fusion protein; SAPK/JNK: stress-activated protein kinase/c-Jun amino-terminal kinase; SP600125: a SAPK/JNK inhibitor; Zn: zinc.

methane dicarboxylic aldehyde production *in vivo* and *in vitro* and that selenium increases the expression of apoptosis-promoting factors caspase-3 and cytochrome c in rat oral squamous carcinoma cells (Qiao et al., 2017). Thus, we believe that detailed effective dose determination and adverse effect dose determination using animal models is important. In addition, several clinical trials have been conducted using selenium-containing compounds, and we must understand the results of those trials. In particular, the effect of selenium on AD, one of the most common neurological diseases, is controversial. For example, a large clinical trial (PREADViSE) examining the efficacy of vitamin E and selenium in preventing AD did not confirm the efficacy of these supplements (Kryscio et al., 2017), while another study reported that under oxidizing conditions, selenium exerted an antioxidant effect on lymphocytes collected from AD patients (Cosin-Tomas et al., 2019). Furthermore, Martini et al. have shown that in a metabolic model of sporadic AD, ebselen (a selenium-containing compound) reversed memory impairment and hippocampal oxidative stress by increasing the activities of antioxidants (Martini et al., 2019). These results are similar to those obtained in our *in vitro* analysis. Thus, we believe that the antioxidant-mediated cytotoxic inhibitory effect of selenium-containing compounds is effective against neurological diseases. However, to demonstrate sufficient efficacy, selenium-containing compounds must be properly delivered to the disease site. In animal models, it has been reported that the administration of nanoparticles encapsulating selenium and flavonoids reduces amyloid, a protein that plays a major role in the development of AD, through the suppression of oxidative stress (Naziroglu et al., 2017). Considering all of these reports, we speculate that targeting optimal amounts of selenium to pathogenic sites may lead to the development of drugs that can inhibit the onset and exacerbation of neurological diseases without causing side effects. We would like to take up these challenges as future research topics.

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