学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Ameriorative mechanism of dietary components on cadmium or mercury-induced toxicities in PC12 cells
（PC12 細胞におけるカドミウムまたは水銀によって引き起こされる毒性に対する 食物成分の改善メカニズム）

Environmental pollution caused by toxic metals receives considerable attention due to the impacts on human health. Recently contaminations of metals (such as mercury (Hg) and cadmium (Cd)) and their associated health risks have been reported extensively and led interest of searching for medicines of metal induced toxicity. In this regard, dietary components can be appropriate therapeutics against metal-toxicity. Essential micronutrients selenium (Se) and zinc (Zn), dietary supplement dihydrolipoic acid (DHLA) and food additive butylated hydroxytoluene (BHT) have been used in this study, to evaluate the protective actions against metals induced harmful effects in PC12 cells. Here, it was hypothesized that "food components have regulatory effects on metals mediated toxicity at cellular level".

In chapter 2, the purpose was to investigate the mechanism/s of cyto-protection by Se (Na2SeO3; Se4+) against Cd (CdCl2; Cd2+)-induced cytotoxicity. In addition, Se (5, 10, 20 and 40 μM)-induced cytotoxicity was determined. Cytotoxicity assays and western blot analyses confirmed that Se (≥10 μM) promotes autophagic cell death via inhibition of mTOR activation and p62 accumulation due to increase of cellular oxidative stress. On the other hand, co-presence of non-toxic Se (5 μM) and toxic Cd (5 μM) showed to increase cell viability, glutathione and glutathione peroxidase 1 (GPx1) levels, and to decrease DNA fragmentation and lactate dehydrogenase (LDH) activity compared to Cd-treated (5 μM) cells alone. Furthermore, the co-exposure of Se with Cd significantly decreases the release of cytochrome c into cytosol from mitochondria, and up-regulates ERK1 protein to inhibit Cd-induced apoptosis. In conclusion, Se (≥10 μM) possess cytotoxicity in PC12 cells; however, co-presence of Se (5 μM) with Cd (5 μM) protects against Cd-induced apoptosis due to inhibition of Cd-induced oxidative stress and subsequently suppression of mitochondrial apoptosis pathway.

In chapter 3, the objectives were to investigate mechanism/s of cytotoxicity of Hg, as well as, the cyto-protection of DHLA against Hg induced toxicity using PC12 cells. Treatment of PC12 cells with HgCl2 (Hg2+) (0-2.5 μM) for 48 h resulted in significant toxic effects, such as, cell viability loss, high level of lactate

dehydrogenase (LDH) release, DNA damage, cellular glutathione (GSH) level decrease and increased Hg accumulation. In addition, protein level expressions of akt, p-akt, mTOR, GR, NFkB, ERK1, Nrf2 and HO-1 in cells were downregulated; and cleaved caspase 3 and cytochrome c release were upregulated after Hg2+ (2.5 μM) exposure and thus inducing apoptosis. However, pretreatment with DHLA (50 μM) for 3 h before Hg2+ (2.5 μM) exposure showed inhibition against Hg2+-induced cytotoxicity by inhibiting Hg accumulation, with alterations of the protein level expressions. In conclusion, DHLA could attenuate Hg2+-induced cytotoxicity through boosting up of antioxidant defense and limiting Hg accumulation.

In chapter 4, the ameliorations of Se counter to Hg-mediated toxicity in PC12 cells was investigated in simultaneous exposure. Cytotoxicity assays have been shown that co-treatment of Se (5 μM) appeared to inhibit intrinsic apoptosis and oxidative stress induced by Hg (5 μM) via boosting GPx and GSH contents, limiting DNA degradation, and reversing protein expressions of mTOR, akt, ERK1, cleaved caspase 3 and cytochrome c. In conclusion, co-treatment of Se attenuates Hg-cytotoxicity through its antioxidant properties.

In chapter 5, the regulatory role of BHT was evaluated against inorganic Hg-induced cytotoxicity by a co-treatment method. BHT (100 μM) and Hg2+(5 μM) co-exposure showed prevention against Hg2+-influenced toxicity by improving cell viability, LDH release, DNA damage, GSH content and protein expressions of akt, mTOR, ERK1, Nrf2, HO-1, cleaved caspase 3 and cytochrome c. In conclusion, BHT inhibits Hg2+-mediated toxicity via enhancing antioxidant defense.

In chapter 6, the effects of Zn on Hg-induced cytotoxicity were investigated. Cells pretreated with Zn2+ (100 μM) before Hg2+(5 μM) -exposure, showed a significant improvement in cell viability, cell membrane, DNA damage, glutathione level and apoptotic cells, with reversal in mTOR, akt, ERK1, nrf2, HO1, bcl2, bcl-xL, p53, bax, cytochrome c and cleaved caspase 3 expressions, indicating inhibition of intrinsic apoptosis by Zn2+-pretreatment. To conclude, Zn2+-pretreatment suppress cytotoxicity and inhibit intrinsic apoptosis induced by Hg.

Heavy metals (Cd and Hg)-exposure even in trace amount, can cause deleterious health effects and diseases in human. However, this research recommended that food components (Se, DHLA, BHT and Zn) could be used as therapeutic agents in combating metal induced effects in biological systems.