平成 30 年度　環境科学院　修士論文内容の要旨

Comparative evaluation of L-ascorbic acid and epigallocatechin gallate on cadmium-induced toxicity on PC12 cells

(PC12 細胞におけるカドミウム誘導毒性に対する L-アスコルビン酸とエピガロカテキンガレートの比較評価)

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Exposure to environmental toxicants, such as heavy metals, is an increasing problem around the world, as these pose a great risk on human health and affect the quality of living of many people. Evidence suggests the correlation between nutrients from human diet and environmental toxicants; however, this field has not been significantly studied. It is of importance to study the interactions of nutrients in the dietary level and toxicants and the possible implications on human health, especially on populations that are of greater risk of exposure and have less access to medical care. Cadmium (Cd), a toxic heavy metal, mainly exposed to humans via metal industries and consumption of tobacco products, has been found to cause various lethal effects on human health by targeting organs, affecting body systems and even inducing cancer. Studies show Cd triggers cellular death by regulating ion homeostasis and redox, but usage of anti-oxidants such as vitamins and phytochemicals can mitigate Cd's cytotoxic effects. This study aims to elucidate the cellular mechanisms of bioavailable concentrations of dietary chemicals against Cd and simulate possible biological effects of such diet on humans exposed to environmental toxicants.

In this study, two common chemicals found in food were tested for their interactions with and without Cd. L-Ascorbic acid (Vitamin C, 80 μM) found mostly in fruits, and (-)-epigallocatechin gallate (EGCG, 1.5 μM) found in tea, were used on PC12 cells which were then treated with 5 μM of Cd. Several procedures were then employed: trypan blue assay to check the cell viability, LDH assay for extent of cell damage, DTNB assay for free sulfhydryl levels, and western blotting for protein marker expression to elucidate cellular pathway.

Cell viability assays showed exposure to 5 μM of Cd significantly decreased the cell viability but addition of vitamin C protected against cell death. On the other hand, combination treatment of EGCG and Cd significantly decreased cell viability compared to Cd alone. EGCG also lowered cell viability upon combination with vitamin C and Cd. LDH assay and free sulfhydryl levels indicated cell membrane damage and oxidative stress in Cd and Cd-EGCG treated cells. Western blotting revealed that the pro-apoptotic proteins, Bax, procaspase-9, p53, and ERK1 were downregulated in cells treated with Cd alone and EGCG-Cd. In contrast to this, pro-cell survival proteins, mTOR and Akt, were upregulated in vitamin C-Cd and vitamin C-EGCG-Cd treated cells. Apart from this, autophagic proteins such as p62, pBeclin1, and pULK1 expressions were upregulated on combination treatments with Cd. These findings indicate that (1) EGCG and vitamin C promote cell survival individually however, (2) EGCG interaction with Cd show possible enhancement of Cd toxicity; and (3) Cd treated cells undergo a non-apoptotic cell death; wherein (4) progression of cell death can be enhanced through autophagy.