学 位 論 文 内 容 の 要 旨

博士（環境科学）　　　　氏　名　Serene Ezra Corpus Bondad

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Effects of environmental factors on pro-oxidant properties of
epigallocatechin gallate
（エピガロカテキンガレートの酸化促進特性に対する環境要因の影響）

Despite being a crucial molecule that sustains life, oxygen is toxic and can induce harmful effects ranging from cell and DNA damages, to mutation and carcinogenesis. Anti-oxidants have played crucial roles in keeping the balance between the benefits and hazards of oxygen. However, when innate anti-oxidants in our system are unable to cope with the levels of oxidation, there is a need to obtain anti-oxidants from external sources like food. Polyphenols, such as epigallocatechin gallate (EGCG) found mostly in green tea, have gained the interest of both researchers and the general public due to its reported health benefits, with its anti-oxidant property being one of the most explored. However, considerable amounts of studies have also shown that EGCG exerts pro-oxidant effects, driving the need to investigate such situations that trigger this pro-oxidant effect. This study aims to explore the pro-oxidant effects of EGCG through the involvement of environmental factors, mainly heat, toxicants, anti-oxidants, and cancer cells. Chapter 2 focuses on the interaction of EGCG with L-ascorbic acid (commonly referred to as vitamin C) under the presence of cadmium (Cd), a known environmental toxicant. EGCG, vitamin C, and its combination does not pose cytotoxicity on PC12 cells. However, when Cd is present, EGCG amplifies Cd toxicity and antagonizes the anti-oxidant property of vitamin C. Membrane damage and depletion of cell anti-oxidant glutathione was also observed to be exerted by EGCG with Cd.

The findings from Chapter 2 show that the combination of two anti-oxidants does not necessarily mean an enhanced effect against a toxicant, rather it can lead to an antagonistic effect. Chapter 3 addresses previous findings that polyphenols like EGCG oxidizes under the presence of metal ions (such as Cd), which explained EGCG’s toxicity when combined with Cd. By inducing EGCG oxidation through heat, prior to Cd co-exposure, it was hypothesized that EGCG-Cd cytotoxicity would decrease. Results have proven the contrary, with heated

EGCG still being able to retain its cytotoxic property when combined with Cd. Reactive oxygen species (ROS) levels, and expression of proteins analyzed by western blot, revealed that unheated and heated EGCG triggered different pathways to induce cell death. The findings from Chapter 3 indicates that the cellular effects of EGCG is not straightforward and rely on its chemical activity, possibly its structure. Chapter 4 focuses on exploring the anti-cancer potential of EGCG on MCF-7 breast cancer cells. As it was proven by several journals that heat can degrade polyphenols, the anti-cancer activity of EGCG on MCF-7 cells was explored through treatment of cells by heated or unheated EGCG. LCMS analysis of EGCG solutions showed a decrease in EGCG peak intensity, showing that heating did cause changes in the polyphenol. MCF-7 cells exhibited a decrease in viability, cell number, and membrane integrity after being treated with EGCG; with heated EGCG solutions having more potency to kill cells. ROS measurement showed that EGCG induced ROS generation in the cells, further supporting the findings of EGCG pro-oxidant behavior. However, ROS amounts and antioxidant catalase depletion was found to be lesser as heating time of EGCG progressed. This indicates that if heating time is extended, EGCG may lose its pro-oxidant ability. In all three chapters, it was found that EGCG exerts its pro-oxidant effect under the presence of the toxicant Cd and also with cancer cells, in both cases heat degradation has minimal to no effect in decreasing its cytotoxicity. Comparing the findings from the three chapters with established studies, it was found that EGCG activity relies on its concentration, accompanying chemical, cell line, and condition (e.g., heat degradation).

The eradication of oxidation has always been the goal of many studies; however, oxidation can also be important especially in the case of cancer cell treatment and elimination of damaged cells. Although the specifics of how and when EGCG switches from pro-oxidant to anti-oxidant (and vice versa) is still yet to be fully understood, the findings of this study shows the importance of understanding EGCG as a pro-oxidant. By understanding how EGCG behaves in certain conditions, researchers can be able to manipulate parameters to bring out its desired effect, pro-oxidant or anti-oxidant alike.