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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation　Anti-allergic activity of bioactive compounds in coffee beans (*Coffea arabica* L.)
(コーヒー (*Coffea arabica* L.)豆に含まれる生理活性成分の抗アレルギー効果)

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Coffee is a popular beverage all over the world that has a distinctive taste and aroma. Apart from being consumed as a daily drink, coffee is also believed to have several health benefits. These health benefits are inseparable from the bioactive compounds contained in coffee. Caffeine and trigonelline are major two of the essential compounds in coffee. Besides playing a role in the stimulation of the central nervous system, heart rate, and respiration, caffeine also provides health benefits, such as a source of antioxidants, anti-cancer, anti-bacterial, and anti-inflammatory activities. Meanwhile, trigonelline can be used to inhibit the invasion of liver cancer cells, to prevent dental caries, and to treat hyperglycemia, hyperlipidemia, and kidney dysfunctions. This study aims to determine the anti-allergic activity of these two compounds.

The anti-allergic activity was determined by *in vitro* and *in vivo* experiments by focusing on inhibiting degranulation of mast cells treated with caffeine and trigonelline. This study examined the inhibitory effect of caffeine and trigonelline on β-hexosaminidase release from RBL-2H3 cells that are immunologically induced using dinitrophenyl (DNP)-human serum albumin (HSA) as antigen. The mechanism was investigated from both the Ca^{2+}-dependent signaling pathway and the Ca^{2+}-independent pathway involved in degranulation. The passive cutaneous anaphylaxis (PCA) model mice *in vivo* were conducted by performing local extravasation that was induced through a local injection of IgE antibody, followed by a DNP-HSA

antigen challenge.

The inhibitory effect of caffeine on antigen-stimulated degranulation was performed and caffeine was found to be able to inhibit antigen-stimulated degranulation by rat basophilic cell line RBL-2H3 cells without cytotoxicity. Whereas caffeine suppressed the $[Ca^{2+}]_i$ in RBL-2H3 cells induced by antigen in a dose-dependent manner. Caffeine inhibited FcεRI-mediated intracellular signaling pathways by suppressing the phosphorylation levels of Syk, Btk, PLCγ1, PI3K, and Akt kinase in antigen-stimulated RBL-2H3 cells. Degranulation suppression was also provoked by the inhibition of microtubule formation.

By *in vitro* study, it showed that trigonelline inhibited the release of β-hexosaminidase from antigen-induced RBL-2H3 cells. Trigonelline suppressed the degranulation in a dose-dependent manner without cytotoxicity. The effect of trigonelline was not caused by inhibition of antigen-antibody interactions but was caused by modulation of intracellular signaling pathways involved in degranulation in basophil and mast cells. FcεRI-mediated intracellular signaling pathways were inhibited by trigonelline through the phosphorylation levels suppression of PLCγ1, PI3K, and Akt kinase in antigen-stimulated RBL-2H3 cells. The effect of trigonelline on microtubule formation was observed by fluorescence microscopy. The result suggested that trigonelline suppresses degranulation by not only affecting microtubule formation, but also the elevation of intracellular calcium concentration.

In addition, the anti-allergic effect of caffeine and trigonelline was examined using PCA model mice. The intensity of the blue dye was decreased in the mice that were orally administered with caffeine and also trigonelline. Caffeine at a dose of 1 mg/kg body weight (BW) inhibited the PCA reaction 64.1% compared with the control group. While at a dose of 5 mg/kg BW, the inhibition was 49.7% compared with the control group. Meanwhile, trigonelline at 6 mg/kg BW inhibited the PCA reaction 45.7% compared with the control group, while the dose of 30 mg/kg BW inhibited the reaction 59.5% compared with the control group.

In general, caffeine and trigonelline inhibited degranulation of IgE-sensitized basophil cell line by attenuating both the intracellular Ca^{2+}-dependent and Ca^{2+}-independent pathways. Both of them inhibited degranulation by similar mechanism. The similarity in the mechanism of inhibition is likely because both are alkaloids that have almost identical molecular structures.