（学位第３号様式）

学 位 論 文 要 旨

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題　目	The chemopreventive activities and molecular mechanisms of loquat (*Eriobotrya japonica*) tea （ビワ茶の生体調節機能および分子機構に関する研究）

Loquat tea is called “Biwa Cha” in Japanese which is made from loquat leaves roasted to 350 ºC and it is now using for health beverage in Japan. Although accumulated data revealed that fresh loquat leaves have benefit to human health such as chemoprevention and treatment for chronic inflammatory diseases, the bioactivity of roasted loquat leaves (loquat tea) is unknown yet. Therefore, I chose loquat tea as my research objective to clarify the antioxidant, anti-inflammatory and anticancer activities and underlying molecular mechanisms in the present study.

First of all, several types of column chromatography were used to separate the bioactive fractions of loquat tea by antioxidant activity-guided fractionation. The antioxidant potency of the ethanol fractions of loquat tea extract (LTE) and fresh loquat leaves (FLL) was determined by oxygen radical absorbance capacity (ORAC) and DPPH radical scavenging activity assays. Moreover, LTE increased total antioxidant activities *in vitro* by suppressing cellular reactive oxygen species (ROS) in murine RAW 264.7 cells and increasing nuclear factor-erythroid 2 p45-related factor 2 (Nrf2)-mediated expression of heme oxygenase-1 (HO-1).

Since antioxidant activities are linked to anti-inflammatory activities by modulating signal transduction pathways, I next clarified the anti-inflammatory effects and molecular mechanisms of LTE in both cell and animal models. LTE inhibited the production of pro-inflammatory factors including cyclooxygenase-2 (COX-2), prostaglandin E_2 (PGE_2), inducible nitric oxide synthase (iNOS), nitric oxide (NO), interleukin 6 (IL-6), RANTES and tumor necrosis factor (TNF-α). Cellular signaling data revealed that the downregulation of TGF-β-activated kinase (TAK1)-mediated mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) pathways as well as interferon regulatory transcription factor 3 (IRF3) pathway were involved in the inhibition of pro-inflammatory factors by LTE. Animal experiment data confirmed the *in vivo* anti-inflammatory effects of LTE by attenuating LPS-induced mouse paws edema and serum cytokines level.

Because of the bioactive compounds possessing the antioxidant and anti-inflammatory activities will also have anticancer activity, the antiproliferation and apoptosis induction activities of LTE were investigated using human promyelocytic leukemia cells (HL-60). LTE suppressed the proliferation of HL-60 cell and inducing apoptosis by releasing proapoptic proteins from mitochondria membrane loss ($\Delta\Psi m$).

Finally, the bioactive compounds contributing to the antioxidant and anti-inflammatory and chemopreventive activities in LTE were analyzed with the methodologies of high performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy (FT-IR), mass spectroscopy (MS) and nuclear magnetic resonance (NMR). The results revealed that the original flavonoid in fresh loquat leaves such as 3-caffeonylquinic acid, 5-caffeonylquinic acid, epicatechin and procyanidin B2 were reduced in LTE. In place of them, several kinds of new phenolic compounds were detected in LTE, that are produced from the release and/or degradation of bound phenolic compounds from fresh loquat leaves during roasting process.

In summary, LTE possessed stronger antioxidant activity, and could suppress the production of inflammatory mediators by downregulating inflammatory signaling pathways *in vitro* and *in vivo*. LTE also suppressed the proliferation of HL-60 cell by inducing mitochondrial dysfunction pathway. The results provide insight for understanding chemopreventive effects and molecular mechanisms of loquat tea.