論文概要

(Summary of the Thesis/Dissertation)

Doctoral Program in Life Science Innovation
School of Integrative and Global Majors
University of Tsukuba

Course: **Disease Mechanism**

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Title of the Thesis/Dissertation : The Bioactivities in Honeybee Propolis: Molecular Analysis of Anti-stress and Anti-cancer Activity

2. Summary (1,000 - 1,200 words in English)

Propolis is produced by honeybees from materials collected from plants they visit. It is a resinous material having mixtures of wax and bee enzymes. Propolis is also known as bee-glue and used by bees as a building material in their hives, for blocking holes, cracks, repairing the combs and strengthening their thin borders. It has been extensively used since ancient times for different purposes in traditional human healthcare practices. The quality and composition of propolis depends on its geographic location, climatic zone and local flora. The New Zealand and Brazilian green propolis are the two main kinds being extensively studied in recent years. Their bioactive components have been ascribed to possess a variety of therapeutic potentials. It was found that Brazilian green propolis improves the cognitive functions of mild cognitive impairment in patients living at high altitude and protects them from the neurodegenerative damage through their antioxidant properties. It possesses Artepillin C (ARC) as the key component, also known to possess anticancer potential. The New Zealand propolis contains Caffeic Acid Phenethyl Ester (CAPE) as the main bioactive with multiple therapeutic potentials. CAPE has been assigned several kinds of bioactivities of which anticancer and antistress activities have been demonstrated in cell culture and mice assays in our laboratory. In contrast to New Zealand propolis, Brazilian propolis has not been studied well in the laboratory and the effect of its main bioactive component (ARC) has not been well evaluated with respect to CAPE. I am currently using CAPE and ARC for my research to investigate (i) their anticancer, anti-stress and hypoxia-modulating activities, and (ii) their mechanism(s) of action and (iii) their potential as Natural, Economic & Welfare (NEW) anticancer and antistress drug.

In the first study, I used the extract prepared from Brazilian propolis (Green Propolis Supercritical Extract- GPSE) and tested their cytotoxicity in human cancer cells. Its known active ingredient, Artepillin C (ARC- 3,5-diprenyl-4-hydroxycinnamic acid) that has been shown to possess various biological activities (anti-viral, anti-bacterial, antioxidant, and anti-carcinogenic) was used in parallel. Bioinformatics and experimental evidence revealed that the ARC, similar to CAPE, targets mortalin-p53 interactions, resulting in nuclear translocation and reactivation of p53 function leading to growth arrest in cancer cells. However, its cytotoxic efficacy was low. GPSE, on the other hand, showed higher efficacy *in vitro* toxicity assays. Green propolis super-critical extract (GPSE) containing 9.6% of Artepillin C was cytotoxic to cancer cells with a dose ranging from 0.25% to 0.5% (containing 8.3 μM to16.6 μM of Artepillin C). We anticipated that this effect could be due to the presence of multiple bioactive compounds including a variety of flavonoids and antioxidants. Based on this data, GPSE was selected for further analysis. I investigated the dose dependent effects of GPSE extract on cancer cell proliferation, migration, invasion, protein aggregation and hypoxia signalling. I found that the low doses of GPSE lack cytotoxicity, and cause activation of anti-stress signalling that was in turn mediated through deaggregation of misfolded proteins, protection from H_2O_2-induced mitochondrial stress and activation of differentiation, hypoxia and autophagy. Molecular analysis of these phenotypes was undertaken at the protein level wherein I examined the level of expression of several proteins by Western blotting and immunocytostaining using specific antibodies. Differentiation of C6 cells by ARC and GPSE was compared with

Ashwagandha bioactives (Withaferin A and Withanone) that have been reported to cause astrocytic, neuron and muscle differentiation. In view of the current pandemic, I extended the analysis of Propolis components (CAPE and ARC) for anti-covid activity using computational and experimental approaches. Docking capacity of ARC and CAPE for viral protein, M^{pro} that is essential for viral replication and human cell surface receptors (ACE2 and TMPRSS2) that are essential for virus infection was examined by molecular docking. Expression of ACE2 and TMPRSS2 mRNA and protein in control and treated cells was examined by RTqPCR, Western blotting and Immunocytostaining with specific antibodies. In this analysis, I compared the response with Ashwagandha bioactives that have been shown to possess remarkable anti-covid potential.

In order to address further the mechanism of action of GPSE of which many of the activities could be assigned to its antioxidant activity, I selected alpha-lipoic acid (ALA), a potent thiol antioxidant and mitochondrial metabolite. It is a natural antioxidant found in all kinds of cells and has been shown to possess several physiological functions. It is widely used as medicine as well as dietary supplement. ALA is readily absorbed by cells and reduced to dihydrolipoic acid (DHLA) that has been shown to quench reactive oxygen species (ROS), reduce oxidative stress, and recycle other antioxidants including vitamin C, vitamin E and glutathione. Several studies have reported that it causes substantial increase in reduced glutathione and plays a central role in the regulation of tissue antioxidant defenses and redox signaling mediated by NF-kB. An inhibitory effect of ALA on the pro-inflammatory cytokine interleukin-6, NF-kappaB-p65, COX-2, TNF-alpha, IL-6, MMP-9 and MDA accumulation has been reported. Studies in rodent models of oxidative stress have shown that ALA supplementation offered protection against reactive oxygen species, oxidative lipid tissue damage, oxidative DNA damage and improved the antioxidant status. Dietary supplementation of R-lipoic acid for two weeks was shown to cause complete reversal of the age-related decline in hepatocellular GSH levels and increased vulnerability to t-BuOOH. I investigated antistress activities R-Alpha lipoic acid (R-ALA) and S-Alpha Lipoic acid (S-ALA) on cell culture models of a variety of stresses and differentiation using brain derived cells. Dose dependent response of cells to R-ALA and S-ALA was determined using MTT, WST and QCV assays established in our laboratory earlier. Molecular investigation on the effects of R-ALA and S-ALA on stress-induced protein deaggregation, misfolding and differentiation and H_2O_2-induced mitochondrial stress assays were compared with GPSE and ARC. I found that low non-toxic doses protect from these stresses. Furthermore, culturing of normal human lung fibroblasts in R-ALA and S-ALA supplemented culture medium extended their *in vitro* lifespan. The differentiation inducing potential of R-ALA and S-ALA was examined using brain derived cells. Molecular analyses of differentiated cells are underway. It is anticipated that GPSE, ARC, R-ALA and S-ALA possess anti-stress activities and promote neuro-differentiation suggesting their potential in management of brain pathologies.