

Studies on Antiobesity-related Compound in Okinawan Medicinal Plant *Peucedanum japonicum* Thunb: Identification and its Mechanisms of Action

Obesity and overweight are major health concerns worldwide due to their contribution in numerous metabolic disorders. Previous studies on *Peucedanum japonicum* Thunb (PJT) have reported anti-obesity properties available in PJT powder. However, PJT as a crude extract restricted in-depth investigations on mechanisms related to anti-obesity. In this thesis, partial purification of PJT, isolation of the active compound responsible for anti-obesity 'pteryxin' in PJT and its characterization were investigated.

The first experiment examined the anti-obesity activities of partially purified fractions of PJT *in vitro*. This study focused on the lipid accumulation in 3T3-L1 adipocytes, HepG2 hepatocytes and glucose consumption in C2C12 muscle cells. The gene modulation pattern in each cell type due to ethanol extract (EE), hexane phase (HP) and water phase (WP) of PJT was examined. The HP significantly downregulated lipogenic gene expressions in hepatocytes, inhibited TG accumulation, and decreased the size of 3T3-L1 adipocytes. In C2C12 myotubes, HP tended to enhance energy expenditure. The results suggested that PJT HP possessing the characteristics of anti-obesity during the partial purification.

The second experiment isolated the active compound from PJT HP responsible for anti-obesity, namely pteryxin, a previously known coumarin in PJT. The dose dependent effect on the triglyceride (TG) content, and the gene expressions related to adipogenesis, energy expenditure and lipogenesis due to pteryxin were examined *in vitro*. Pteryxin dose-dependently suppressed TG content in both 3T3-L1 and HepG2 cells. Key lipogenic transcription factors were downregulated in pteryxin-treated 3T3-L1 and HepG2 cells. The energy expenditure was upregulated due to pteryxin. This study demonstrated that pteryxin in PJT play the key role in regulating lipid metabolism related gene network *in vitro*.

Next, the efficacy of pteryxin containing PJT extracts against obesity in C57BL/6 mice fed a high-fat diet was assessed. The PJT EE was fractionated into HP and WP. The pteryxin content in each fraction was quantified by HPLC. The pteryxin-rich HP and EE attenuated the body weight of mice after 4 weeks. HP suppressed blood glucose, TG content and showed a downregulatory trend in insulin resistance. The highest marked reduction in epididymal white adipose tissue weight was observed due to the HP diet. The TG formation and storage in liver were reduced. This study demonstrated that pteryxin in HP plays an important role in regulating lipid metabolism-related gene expression and energy expenditure *in vivo*.

Finally, I investigated the molecular mechanisms of pteryxin in attenuating adipogenesis. Pteryxin was used at low (20 $\mu\text{g}/\text{mL}$) and high (50 $\mu\text{g}/\text{mL}$) doses in 3T3-L1 cells either during the entire adipogenesis or only during the proliferation stage and performed next generation sequencing (NGS), qPCR, and protein expressions related to different doses of pteryxin. I found that pteryxin at two different doses attenuate adipogenesis, however, pteryxin at 20 $\mu\text{g}/\text{mL}$, downregulated lipogenic genes and increased lipolysis. The 50 $\mu\text{g}/\text{mL}$ pteryxin completely suppressed adipocyte differentiation in 3T3-L1 adipocytes. The NGS data analysis suggested that pteryxin at 50 $\mu\text{g}/\text{mL}$ downregulated Wnt5a non-canonical pathway. I found that ERK1/2, AMPK activities play a major role in the adipogenesis inhibition in the presence of 20 and 50 $\mu\text{g}/\text{mL}$ pteryxin, respectively.

In conclusion, these results provide great insight on the unique characteristics of pteryxin in the anti-adipogenic process in order to utilize pteryxin as an anti-obesity drug in the pharmaceutical industry in the near future.