Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Discovery of Natural Sphingomyelin Synthase Inhibitors against High Fat Diet-Induced Obesity and its Lipid Metabolism in Mice

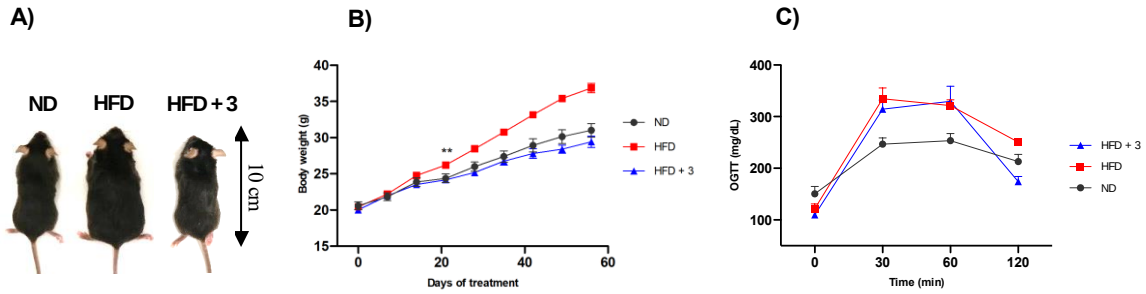
(マウスにおける高脂肪食誘発肥満に対する天然スフィンゴミエリン合成酵素阻害剤の発見とその脂質代謝)

Worldwide prevalence of obesity has increased substantially over the past 40 years and continues to cause metabolic syndrome which is associated with dyslipidaemia, insulin resistance, cardiovascular diseases and type 2 diabetes mellitus (T2DM). These intersecting risks are controlled by a critical and complex metabolic pathway which involves the membrane protein. Having said that, the membrane protein could be the initial key in enhancing the understanding of pharmacology for common metabolic related diseases, notably, obesity. The membrane protein regulates cell communication with their surroundings which is activated by a wide variety of physiological and environmental stimuli including peptides, proteins, small organic molecules, and even ions. About more than 50% of all known low molecular drugs bind to the membrane protein. Thus, discovering an enzyme inhibitor will be a direct approach in developing low molecular drugs.

This study of ours focuses on the sphingomyelin synthase (SMS) membrane protein family which consists of two isozymes, SMS1 and SMS2. The SMSs modulate SM and other sphingolipids levels, thereby regulating membrane fluidity, ceramide-dependent apoptosis, lipid metabolism and signal transduction. The increasing levels of SM and DAG produced by the SMSs will lead to obesity and insulin resistance. SMS knockout mice are resistant to Alzheimer's disease, tumorigenesis, diet-induced obesity, T2DM and are also know to exhibit decreased levels of plasma inflammatory cytokines. Therefore, the inhibition of the SMSs enzymes by natural occurring substrates would be an ideal therapeutic approach for metabolic syndrome.

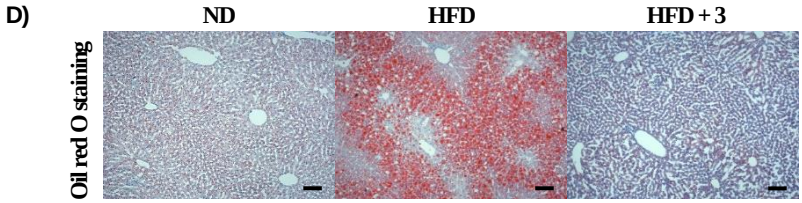
Very recently, the inhibitory activity of gingkolic acid from the leaves of *Gingko biloba* was reported. Though, gingkolic acid has been proven to be an effective inhibitor with equal inhibiting potentials (IC_{50} = 1.5 μM) against both enzymes, studies have revealed that gingkolic acid is toxic, thus making it an unsuitable candidate for the further development of it as a drug. With regard to this, in the present work, I decided to investigate the fruits of *Myristica cinnamomea* King from Malaysia in an effort to discover potential SMS1 and SMS2 inhibitors which at the same time do not display any harmful side effects. Herein, I succeeded in the isolation of malabaricones A-C, E (**1**-**4**) as the first naturally occurring SMS inhibitors from the edible plants. Acute toxicity studies of malabaricone C (**3**) were previously conducted on mice liver and kidneys. The absent of inflammation, necrosis and haemorrhaging in the respective organs further supported my findings. These results suggested that malabaricone C (**3**) could be a suitable candidate for further *in vitro* and *in vivo* studies based on its previously reported world drug index, Lipinski's rules, non-mutagenicity and non-carcinogenicity.

Having the same mechanisms of action as the previously reported SMS knockout studies, malabaricone C (**3**) was highly efficacious in preventing oleic acid uptake across the membrane which in turn reduced lipid droplet formation *in vitro*. Malabaricone C (**3**) was also found to be able to significantly reduce body weight gain, improve glucose tolerance *in vivo*, thus making this the first report involving a plant derived SMS inhibitor against high fat diet-induced obesity.



A) Representatives images of the whole mice body; B) Body weight gain; C) Oral glucose tolerance test

Previous study has shown that up-regulation of the hepatic lipid metabolism may contribute to the suppression of the liver fat and visceral fat accumulation. Examination of the histological analysis of the liver tissues showed the presence of numerous steatosis in the HFD control group. The HFD + **3** group on the other hand exhibited resistance in the development of liver steatosis and improved lipid metabolism.



D) Representatives images of oil red O staining (*N* = 3 mice per group)

Consistent with the histochemical results, I found that HFD + **3** effectively reduced the hepatic TG levels. In addition, feeding the mice with HFD + **3** significantly reduced the levels of triglycerides (TG) and free fatty acids (FFAs) in the blood plasma. In comparison with previous plasma free fatty acids in the SMS2 knockout mice *in vivo*, there is a possibility that the uptake of fatty acids into the liver tissues may not fully be prevented which further explains the decrease of plasma free fatty acids upon feeding with HFD + **3**.

Despite having different results in lipidomics analysis, I assessed the synthesis of DAG and SM via liver tissue lysate assays to further confirm the *in vivo* SMS inhibitory activities by malabaricone C (**3**). Indeed, I have proved that for the first time, malabaricone C (**3**) as a natural SMS inhibitor, has significantly reduced the synthesis of the DAG and SM in the liver. Furthermore, screening of other medicinal plants identified 9 other promising natural SMS inhibitors among which one of them is a fatty acid. Due to that, a series of fatty acids which consist of saturated and unsaturated fatty acids were also being evaluated.

As a result, liver lipid analysis revealed a crucial link between the SMS activities of malabaricone C (**3**) and its lipid metabolism *in vitro* and *in vivo*. The non-toxic nature of malabaricone C (**3**) makes it a suitable candidate for its further development as a new drug or medicinal supplement to treat and prevent obesity.