Abstract of Master's Dissertation

No.1

Course	Health Innovation (MSc)	Name	Quankai Mu
Thesis Title	Insights into Anti-Trypanosomal Mechanisms of Coptisine derived from Kampo Medicine		

Abstract of Master's Dissertation

Background: Chagas disease, also called American trypanosomiasis, is caused by the protozoan parasite *Trypanosoma cruzi*. About 6–7 million people worldwide, mainly in Latin America, are estimated to be infected with the parasite. There are two phases of infection, acute and chronic. After the invasion of infection from the parasite, intracellular propagation of the parasite occurred locally. The acute phase persists for one to two weeks and is usually transected to the chronic phase characterized by asymptomatic and systematic persistent infection. Currently, two widely used anti-*T. cruzi* drugs, nifurtimox and benznidazole, are effective in the acute phase but not the chronic phase. When used for chronic, significant side effects and variable efficacy are observed. Therefore, there is an urgent need to produce new, effective drugs to treat Chagas disease. One of the efforts to find a seed medicine is to use a resource of traditional herbal medicine, which we call Kampo in Japan. In previous studies, coptisine, one of the main protoberberine-type alkaloids of Coptidis Rhizoma, has been shown anti protozoan parasites activity, including anti-malaria and anti-*T. cruzi* potential.

Objectives: In this study, we investigated the possible mechanisms of coptisine on the anti-trypanosomal effect *in vitro*

Methods: The possible molecular targets of coptisine and the drug-responsible metabolites profile are assessed with the Agilent Seahorse Extracellular Flux Technology and the capillary electrophoresis-mass spectrometry analysis. The Agilent Seahorse Extracellular Flux technology allows examining real-time changes in oxygen consumption rate due to ATP turnover, proton leak, and electron transfer capacity to assess mitochondrial respiratory function in living cell. Due to the addition of a proton (H^+) detection probe, independently senses the pH in each well of the plate to measure extracellular acidification rate in test *T. cruzi* cells, making it possible to investigate changes in glycolytic as well as oxidative metabolic flux. At the same time, the four ports in the assay cartridge above each well can inject different drugs to detect quantitative changes in oxygen consumption rate and extracellular acidification rate. The capillary electrophoresis–mass spectrometry, the combination of the liquid separation process of capillary electrophoresis with mass spectrometry, is an analytical chemistry technique for the efficient profiling of polar and ionizable compounds in the given biological samples, notably for

* The abstract, containing background, objectives, methods, results and conclusion should not exceed 300-500words and printed double sided on A4 paper)

Abstract of Master's Dissertation

Course	Health Innovation (MSc)	Name	Quankai Mu

compound classes such as nucleotides, sugar phosphates, organic acids, nucleotides, and amino acids. This approach has been successfully applied for the comprehensive profile of intracellular metabolites under various conditions, including drug treatment and parasite infection.

Results：In the extracellular flux assay, using oligomycin A, a classical bc_1 complex (or complex III) inhibitor, as a control, coptisine and benznidazole (reference compound) showed any effect on mitochondrial respiration, as indicated by unaffected oxygen consumption rate, whilst oligomycin A completely inhibited parasite respiration. Interestingly, coptisine presented an inhibitory effect on the extracellular acidification rate which was not observed in benznidazole. While using glucose, pyruvate and glutamine as carbon sources to probe possible target metabolic pathways, it could still stably and similarly reduce extracellular acidification rate in multiple experiments without causing changes in oxygen consumption rate. This result was consistent with the intracellular metabolomics profiles, which showed no significant difference in the production of various metabolites of mitochondrial respiration and glycolysis between treated and untreated groups with coptisine. In total, 379 intracellular metabolites were successfully detected by capillary electrophoresis–mass spectrometry which may relate to 11 metabolic pathways including carbohydrate metabolism, glucose metabolism/gluconeogenesis, energy storage/conversion by TCA, glutamate metabolism, choline metabolism, nucleic acid metabolism, aromatic amino acid metabolisms (tryptophan metabolism, phenylalanine metabolism, tyrosine metabolism). Among these, 7.1% of metabolites (27/379) altered significantly in response to the coptisine treatment. Interestingly, the glycerophospholipid synthesis pathway, including choline, phosphorylcholine, ethanolamine phosphate, and glycerophosphocholine, is profoundly affected by coptisine treatment, implying a unique metabolic feature in the anti-trypanosomal effect of this compound.

Conclusion：In this report, we investigated the inhibition efficiency and the possible mechanism of action of coptisine against *T. cruzi* using extracellular flux technology and metabolomic analysis. We found that coptisine significantly inhibits the *T. cruzi* growth *in vitro*. A significant effect on the extracellular acidification rate and the glycerophospholipid synthesis of the parasites indicated the possible drug targets on the energy and lipid metabolism of cells. Our works provide new insights into the development of anti-trypanosomal drugs and the research of therapeutic targets.