Application of NMR metabolomics in understanding the physiological target of Kampo medicine

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Nuclear Magnetic Resonance (NMR) Spectroscopy is an indispensable tool for the structure elucidation of the compounds in natural products and organic chemistry. Recently, NMR is also becoming a major technique for the analysis of metabolites in bio-samples, and the biomarker discovery. A key advantage of NMR metabolomics is its high reproducibility and direct identification of the metabolites. Furthermore, it is a non-destructive technique, which can rigorously quantify major compounds present in both in vitro and in vivo bio samples. Given its simplicity of methodology, direct identification and quantitative information of the metabolites, we applied NMR metabolomics as a novel and powerful tool to determine the physiological target of the Kampo medicine in vivo. For this purpose, a Kampo medicine 'Gorei san' (五苓散) was used as the exploratory example in this study.

Gorei san (GRS)is one of the traditional Kampo medicine composed of five crude drugs (Alisma Rhizome, Chuling (猪苓), Poria Sclerotium (茯苓), Cassia (桂皮) and Atractylodes Rhizome (白朮). It is regarded as a typical water-retaining agent, and it has the function of regulating the metabolic abnormality of moisture in the body and returning it to normal. In the present study, male Wistar rats of 8 weeks of age were fed for four weeks with the regular diet (Cont group, n = 6) or a feed containing 1% GRS extract (1% GRS group, n = 6). Measurement of food intake, drinking water, body weight and urine volume was monitored each week. After the administration period, various organs and bio-fluids such as blood and urine were collected for the NMR analysis. In the present investigation, 26 primary fluctuating metabolites were identified and quantified. Administration of GRS was found to significantly alter the metabolites involved in pyruvate metabolism pathways, such as lactate, acetate, succinate, and citrate.

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Japanese Herbal Medicine (Kampo) Based Antimalarial Drug Development

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Introduction: Malaria is critical global health issue especially tropical and subtropical countries. The emergence of resistance to the available antimalarial drug requires the urgent development of new medicine with new mechanisms of action. Herbal medicine, including Japanese (kampo) one, is still an attractive source of the antimalarial drug since the isolation of quinine and artemisinin. The aim of the study is to examine kampo as the source of new antimalarial drug utilizing a compound and crude extract library. In previous joint seminar, we showed our finding of antimalarial kampo compounds and crude drug extract. According to the finding, in parallel with the compound based drug development strategy, we sought a way of drug discovery based on already approved kampo formula which contains the effective kampo crude extract because it is proven to be enough safe.

Methods and Material: As reported in previous seminar, the antimalarial activity of 96 compounds and 120 extracts in the library provided by Institute of Natural Medicine, University of Toyama were evaluated using *in vitro* antimalarial assay against chloroquine/mefloquine sensitive (3D7A) and -resistant (Dd2) strain of *Plasmodium falciparum* in erythrocytic cycle. After the drug treatment, the red blood cells were stained with SYBR Green to detect the parasite. In addition, the cytotoxicity was also examined. We select the kampo formula based on the content of active crude drug extract, and evaluated using the *in vitro* assay. Moreover, active kampo crude drug extract and formula were further examined against mouse malaria *P. yoelii*. *In vivo* parasite growth suppression by them were evaluated in mice inoculated with parasite (1×10^4/mouse). This *in vivo* antimalarial effect was examined in a 5-day suppressive test (drug treatment started 48 hr before and continued until 48 hr after challenge infection).

Result: As the result of *in vitro* assay with the library, coptisine chloride (compound, IC_{50}=1.1 μM, SI (selective index) =37.8) and coptise rhizome (crude drug extract, IC_{50}=5 μg/mL, SI>200) showed high antimalarial activity and its safety. Then, we examined two approved kampo formula, Sanohshashinto and Ohrengedokuto, containing coptis rhizome by the *in vitro* assay, and those exhibited good antimalarial activities (IC_{50} 36 and 40 μg/mL, respectively). Moreover, even in a preliminary experiment, both Ohrengedokuto and coptis rhizome showed a suppression of parasitemia in malaria mouse model.

Conclusion: High antimalarial activity with low cytotoxicity was observed from kampo formula which contain antimalarial kampo compounds and crude drug extract *in vitro* experiments. Furthermore, even as preliminary experiment, antimalarial effect of kampo crude drug extract and formula were observed *in vivo* experiment, suggesting kampo formula as a potential antimalarial drug. This study is an important input for the development of an antimalarial drug from kampo.