Abstract of Dissertation submitted by
Awet Alem Teklemichael

Title: Anti-malarial activity of traditional Kampo medicine *Coptis* rhizome extract and its major active compounds

漢方生薬オウレンとその主要活性化合物の抗マラリア活性

Awet Alem Teklemichael, Shusaku Mizukami, Kazufumi Toume, Farhana Mosaddeque, Mohamed Gomaa Kamel, Osamu Kaneko, Katsuko Komatsu, Juntra Karbwang, Nguyen Tien Huy and Kenji Hirayama

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Department of Infection Research,
Nagasaki University Graduate School of Biomedical Sciences

Supervisor：Professor Shinjiro Hamano, MD, PhD

Background

Herbal medicine has been a rich source of new drugs exemplified by quinine and artemisinin. In this study, a variety of Japanese traditional herbal medicine (‘Kampo’) were examined for their potential anti-malarial activities.

Methods

A comprehensive screening methods were designed to identify novel anti-malarial drugs from a library of Kampo herbal extracts (n = 120) and related compounds (n = 96). The anti-malarial activity was initially evaluated *in vitro* against chloroquine/mefloquine-sensitive (3D7) and-resistant (Dd2) strains of *Plasmodium falciparum*. The cytotoxicity was also evaluated using primary adult mouse brain cells. After being selected through the first *in vitro* assay, positive extracts and compounds were examined for possible *in vivo* anti-malarial activity. In addition, to analyse the presence of major components derived from *Coptis* rhizome, mice plasma treated with *Coptis* rhizome and Orengedokuto were analysed by LC-MS.

Results

Out of 120 herbal extracts, *Coptis* rhizome showed the highest anti-malarial activity (IC50 1.9 µg/mL of 3D7 and 4.85 µg/mL of Dd2) with a high selectivity index (SI) > 263 (3D7) and > 103 (Dd2). Three major chlorinated compounds (coptisine, berberine, and palmatine) related to *Coptis* rhizome also showed anti-malarial activities with IC50 1.1, 2.6, and 6.0 µM (against 3D7) and 3.1, 6.3, and 11.8 µM (against Dd2), respectively. Among them, coptisine chloride exhibited the highest anti-malarial activity (IC50 1.1 µM against 3D7 and 3.1 µM against Dd2) with SI of 37.8 and 13.2, respectively. Finally, the herbal extract of *Coptis* rhizome and its major active compound coptisine chloride exhibited significant anti-malarial activity in mice infected with *Plasmodium yoelii* 17X strain with respect to its activity on parasite suppression consistently from day 3 to day 7 post-challenge. The effect ranged from 50.38 to 72.13% ($P < 0.05$) for *Coptis* rhizome. The *in vivo* malarial suppression test of coptisine chloride in mice infected *P. yoelii* 17X strain significantly suppress the parasitemia. The parasitaemia suppression (%) of mice treated with coptisine chloride showed significant anti-malarial activity consistently throughout the entire test period to that of the negative control and ranged from 81 to 89% ($P < 0.01$) for coptisine chloride.. After oral administration of *Coptis* rhizome, and Orengedokuto, the signal of berberine, palmatine, and coptisine were detected. The signal of coptisine was relatively lower than that of palmatine and berberine.

Discussion

Since *P. falciparum* has quickly acquired resistance against currently available all anti-malarials, it is urgently required to develop novel anti-malarial drugs. Here it is found that *Coptis* rhizome and its three chlorinated compounds (coptisine, berberine, and palmatine), which are related to *Coptis* rhizome exhibited anti-malarial activity. These compounds belong to the berberine alkaloidal family and share the same isoquinoline skeletons, which is similar to quinoline skeleton found in anti-malarial drug quinine. This structural similarity to quinine is an important indicator of their antimalarial activity. Based on the result of the *in vivo* antimalarial assay, *Coptis* Rhizome at the dose of 122mg/Kg body weight demonstrated a significant antimalarial activity relative to the negative control with regard to parasite reduction (*P-value* < *0.05*) and it suppresses the parasite > 50% from day 3 to 7, suggesting a good antimalarial activity and reported the first time. This in vivo assay confirms the potential antimalarial activity of coptisine chloride, one of the major bioactive components of *Coptis* Rhizome The *in vivo* results of coptisine chloride remarkably suppress the parasitaemia of greater than 80% and the density of parasitaemia was significantly lower than the negative control (*P* < *0.01*). As previously reported, coptisine had wide verities of activities, however, this is the first report of the coptisine chloride to have an *in vivo* anti-malarial activity. After the oral administration of *Coptis* rhizome and Orengedokuto, coptisine were detected in 1/5 and 3/5 of mice, respectively, and the signal of coptisine in plasma samples were relatively lower than that of berberine. Therefore, these results reflect the contents of these alkaloids in *Coptis* rhizome, which suggest that the poor oral absorption and bioavailability, and fast elimination rate of coptisine.

Conclusion

Coptis rhizome and its major active compound coptisine chloride showed promising anti-malarial activity against chloroquine-sensitive (3D7) and -resistant (Dd2) strains *in vitro* as well as *in vivo* mouse malaria model. Thus, Kampo herbal medicine is a potential natural resource for novel anti-malarial agents.

Notes: Summarize your dissertation with 2 pages of A4 (using 12 point, Times New Roman font, single space. Total number of words should not exceed 1000)