CHAPTER IV

Discussion

Prevention and controlling transmission of malaria parasite from vector to host is very important in the process to control malaria infection. Likewise, antimalarial drugs are more important to eradicate all stages of malaria parasite. Efficient drugs are needed for mass treatment, curing asymptomatic infections, curing relapsing liver stage, and preventing transmission. If malaria parasites become resistant to antimalarial drugs, the control of malaria infection will be very difficult and can be a global problem. It is necessary to study about mechanisms of drug resistance developed by the malaria parasites especially malaria parasites from field, which are largely unknown, and at the same time to develop new antimalarial drugs for using in the future.

The aims of this study are to evaluate the antimalarial drug sensitivity and to identify drug resistance genes in *Plasmodium falciparum* from malaria patients living in Mae-Sariang district area, Mae Hong Son province. The information obtained will be useful for monitoring and planning for antimalarial drug usage in the endemic areas and for the development of effective anti-malarial drugs for malaria therapeutics.

In this study, we collected *P. falciparum* parasite isolates from patients, who live in Mae-Sariang district area in Mae Hong Son province. The sample from patients were collected by medical technicians and were transported for culture at the Department of Biochemistry, Faculty of Medicine, Chiang Mai University (CMU). When blood samples arrived our CMU laboratory, they were washed and put into culture as soon as possible. With this process, we could adapt most of the collected parasite isolates in the standard *in vitro* culture. Once adapted the isolates could be frozen down and recovered as needed. Many times, we found that some unadapted isolates were more fragile in *in vitro* culture process. It is possible that the original parasite samples were weakened during the process of collection from patients, transportation until the samples arrived in the lab which sometimes took up to 12 hours. From this study, it is advised that the parasite isolates are

adapted to *in vitro* culture and kept as stock in liquid nitrogen before they were used for other studies in the future.

To evaluate susceptibility of *P. falciparum* field isolates to antimalarial drugs, 36 *P. falciparum* field isolates were cultured and treated *in vitro* with either PYR, CQ, MQ, DHA, and P218. In this study, we used 3D7, K1, CSL-2, and V1/S *P. falciparum* lab strains as control of parasite isolates. The results shown that all the tested isolates were resistant to PYR and CQ, which are the antimalarial drugs not being used in Thailand for more 10 years. Moreover, the results showed that about 70% of the field isolates were resistant to MQ; antimalarial drug which is used in combination with DHA today. The results agreed with study showing increasing in MQ resistance of *Plasmodium* parasites along the Thai-Myanmar border such as Mae-sot district area in Tak. Despite the reports of artemisinin resistant parasites in Thai-Myanmar border areas, all of our tested isolates were still sensitive to DHA. Like MQ resistance, there are many studies hypothesize that *Pfmdr1*, *Pfmrp1*, and *Pfatp6* might be involved, but their mechanism is still unclear. The situation of artemisinin resistance in the endemic area needs close monitoring since artemisinin and its derivatives are our last resources in fighting against malaria through artemisinin-combination therapy (ACT). If it is really confirmed that parasites in the endemic areas are all resistant to artemisinins, the control and treatment of malaria will be impossible when new antimalarial drugs are still in the pipeline of drug discovery and development.

As another effort to fight *P. falciparum* malaria, researcher team at BIOTEC, NSTDA in collaboration with researchers at Chulalongkorn University, Monash University and London School of Hygiene and Tropical Medicine with funding from the Medicines for Malaria Venture (MMV), have developed a new anti-folate antimalarial compound named P218. P218 is a compound specifically inhibit DHFR enzyme of *P. falciparum* (76). It is very effective against PYR-resistant parasites with low toxicity to human cell lines. P218 is currently in the process of GLP-certified pre-clinical study in animal before submission to study in clinical Phase I study. Our study showed that all the tested parasite isolates showed good efficacy to P218 when compared with PYR. The data is encouraging for P218 development since it is better to know that an antimalarial compound is effective, not only to the lab strain parasites that have been in culture in the

laboratory for many years, but also to the field isolates collected from malaria patients. This is to make sure that parasites in the nature do not possess any unknown mechanisms that complicate and negate the efficacy of the drug during development.

It was confirmed that PYR- and CQ-resistance by *P. falciparum* involve mutations of *Pf*DHFR and *Pf*CRT enzymes, respectively. Our sequencing results confirmed that PYR- resistant parasites contain mutations at *Pfdhfr* gene. Most of them have quadruple mutations (N51I+C59R+S108N+I164L), while 3 isolates contain triple mutations (C59R+S108N+I164L), and 1 isolate contains double mutations (C59R+S108N). Interestingly, their IC_{50} to PYR are at similar level with K1 *P. falciparum* strain that contains DHFR double mutations (C59R+S108N), much lower than the IC_{50} of highly PYR-resistant V1/S *P. falciparum* strain that contains DHFR quadruple mutations (N51I+C59R+S108N+I164L). For CQ resistance, it has been known that the mutation of Chloroquine resistance-related transporter (CRT) at codon 76 from K to T is involved. All of our sequencing results showed CQ resistant isolates contain the mutation of *Pfcrt* gene at amino acid positions 74, 75, and 76 from MNK to IET. Some isolates showed a much higher IC_{50} to CQ than the CQ-resistant lab strain K1. These results showed that the genetic background and basic mechanisms of each isolate contributes to the level of drug resistance. The already identified genes, DHFR and CRT, may not be a sole factor for drug resistance. Studies to discover other factors that influence or rescue resistance might be interesting topics in the future.

MQ resistance is of interest in this study because MQ is still using in the field, aiming for *P. vivax* treatment, and might also put drug pressure to *P. falciparum* when the patients are co-infection with the 2 species of malaria. *Pf*MDR1 copy number was reported to be the factor for MQ resistance. In this study, we have not checked the copy numbers of *Pf*MDR1 in each isolate. Among pioneer studies, Price *et al.* had investigated a molecular basis of MQ resistance using *P. falciparum* isolates from uncomplicated malaria patients for 12 years along the northwestern Thai-Myanmar border areas and successfully provided the proof that the parasites with increased *Pfmdr1* copy number are associated with decreased sensitivity to Mefloquine alone or Artesunate-Mefloquine, although the underlying molecular mechanism remains unclear.