Thesis Title Determination of Molecular Mutations in Dihydrofolate Reductase Related to Pyrimethamine-resistance in Falciparum Malaria

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ABSTRACT

To investigate the molecular basis of pyrimethamine resistance in *P. falciparum*, the dihydrofolate reductase (DHFR) gene which encodes the target enzyme was studied by using polymerase chain reaction (PCR) followed by dideoxy sequencing. The nucleotide sequences from four drug-sensitive and seven drug-resistant field isolates and from eight *in vitro* mutagenized drug-resistant parasites were compared. When the nucleotide sequences were deduced to protein sequences, it was found that mutation in codon 108 of serine or threonine to asparagine was the most important change for pyrimethamine-resistance. Additional point mutation of asparagine to isoleucine in codon 51, of cysteine to arginine in codon 59 together with that of isoleucine to leucine in codon 164 were associated with the highest pyrimethamine resistance. In the mutagenized parasites, not only the mutation at codon 108 was found but also mutation to methionine or threonine at codon 164 and even no mutation in DHFR gene. However, mutation at codon 108 was more effective in producing pyrimethamine-resistance than the other mutations. Moreover, parasites having valine at codon 16 and threonine at codon 108 were resistant to cycloguanil (the active metabolite of proguanil). Thus, detection of these mutations in *P. falciparum* DHFR gene may be used to determine the spread of pyrimethamine and cycloguanil resistance in Thailand.