Thesis Title: Mechanisms of Antimalarial Action of Primaquine and Derivatives

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ABSTRACT

The present study has developed a new HPLC technique which is sensitive enough to detect the enzyme dihydroorotate dehydrogenase (DHO-DH) in both asexual intraerythrocytic stage and sexual (gametocyte) stage of the human malaria parasite, *P. falciparum* The column used was C_{18} mBondapak reverse phase. The elution was isocratic with 3 mM PIC A in 5 mM ammonium dihydrogenphosphate containing 5% methanol. This condition

was determined from the appropriate capacity factor and response factor of each component. Under these conditions the substrate, dihydroorotic acid and the products, orotic acid and UMP were clearly separated. It could be demonstrated that the increasing amount of the produact (orotic acid) was coincident with the decreasing amount of the substrate (dihydroorotic acid). Additionally, it also clearly demonstrated that the detected DHO-DH was of parasite origin since there was a linear relationship between the amount of parasite protein, the number of parasites and the percent parasitemia. The intact parasite prepared directly by saponin lysis of parasitized red cells showed the highest enzyme activity compared to sonication and freeze-thawing of the parasites.

Using the developed HPLC method, DHO-DH activity could be demonstrated in all intraerythrocytic stages of *P. falciparum* The ring stage had the lowest activity while the schizont had the highest activity. The DHO-DH activity was reported for the first time in preparations of the gametocytes from culture of *P. falciparum* which showed the level of the enzyme was approximately that found in the asexual trophozoite stage.

This new HPLC technique was also used to study the inhibition of pyrimethamine, primaquine, and WR 238605, a novel primaquine derivative, as well as menoctone, a mitochondrial DHO-DH inhibitor, on parasite DHO-DH at 4h and 24 h of drug exposure. Menoctone was observed to inhibit parasite enzyme synthesis in the absence of parasite killing. Pyrimethamine and primaquine have no effect on parasite DHO-DH inhibition while WR 238605 has

partial inhibition on the parasite enzyme. This conclusion was also applied to the gametocyte culture at 4h drug exposure except primaquine still showed partially effect.

In addition to the evalution of the above conclusion by the detrmination of parasite DHO-DH, fluorescent activated cell sorting (FACS) was also performed. FACS showed a nice linear correlation in determination of parasitemia to the conventional Giemsa'staining thin smears. FACS could also determine the percent parasitemia when the parasites were at the ring stage but with the lower fluorescent intensity compared to the more mature stage. Therefore, the mean values of the fluoresecnt intensity of the parasitized cells showed the clear interpretation while the percent parasitemia showed no difference in the demonstration of inhibition of antimalarials and menoctone. Thus, it needs Giemsa's staining smears for the identification of the stage of parasite.