Mitochondrial genetics in the malarial parasites: Atovaquone-resistant *Plasmodium berghei* as a model

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The development of new antimalarial drug is being a target in combating malaria disease, since the parasites that causes malaria, *Plasmodium*, has developed resistance to the antimalarial drug mainstays, chloroquine and sulfadoxine-pyrimethamine. Studies aimed to identify the molecular mechanism that underlie resistance phenomenon had revealed various mutations in the gene encoding the enzyme target in the malaria parasite.

Mitochondrial inhibitors represent valuable additional chemotherapeutic agents, yet the biogenesis and function of plasmodial mitochondria remain poorly understood. Atovaquone, a hydroxy-1,4-naphthoquinone, is an anti-malarial that shares structural similarity with protozoan ubiquinone, a coenzyme involved in the mitochondrial electron transport. It is effective against chloroquine-resistant strains of *P. falciparum*, and is a major component of Malarone™, a fixed combination of atovaquone and proguanil.

Mutations conferring atovaquone resistance were identified in the mitochondrial cytochrome b gene of *P. berghei*, *P. yoelii*, *P. falciparum*, *Pneumocystis carinii* and *Toxoplasma gondii*. In *Plasmodium* spp., 10 mutations, M133I, L144S, I258M, F267I, Y268C/N/S, L271F/V, K272R, P275T, G280D, and V284F had been documented, mostly located in the quinone binding domain 2 (Qo2). The two main *P. berghei* mutations reported previously by our group, M133I and L144S, were all located in the quinone binding domain 1 (Qo1). To obtain a better model for the biochemical and genetic studies of mutations observed in the human *P. falciparum*, I have now extended the study to isolate a wider range of *P. berghei* resistant strains, in particular those having mutations in the Qo2 region conferring high degrees of resistance. Here I report four new mutations, most in the Qo2 domain, two of which are convergent to codon 268 mutations in *P. falciparum*.

All of those mutations have been proven to associate with resistance to atovaquone to *Plasmodium*.

Technical difficulties in isolating active assayable mitochondria in the malarial parasite hinder us to obtain direct biochemical evidence to support the aforementioned evidence. Following the establishment of the mitochondrial isolation method in the malaria parasite, I further tested the activity of DHO-
cytochrome c reductase in various *P. berghei* atovaquone resistant and sensitive clones in the presence of a wide concentration range of atovaquone. All of the mutant mitochondria showed higher IC₅₀ values (1.45 – 43.5 nM) than that of wild type (0.327 nM). The highest IC₅₀ was found in clones carrying the 268C and 268N mutations with an approximately 100 fold increase.

So far, there has been no report as to how resistance to antimalarial drugs that target function encoded in the mitochondrial DNA (mtDNA) is inherited, while mtDNA is inherited uniparentally through the maternal line. My study of genetic crosses of atovaquone-resistant (atv-r) and -sensitive (atv-s) in murine malaria parasite *P. berghei* indicates that the presence of the mutation in the cyt *b* gene in the atovaquone-resistant *P. berghei* results in the loss of fitness of the parasite during the development of the sexual stage in the mosquito vector. The observation that atovaquone resistance mutation in *P. berghei*, particularly that convergent to *P. falciparum*, affects both growth in erythrocytes and mating fitness might explain the defect in the development in mosquito. This probably leads to observed reduction in transmission of the drug resistance to offspring. My results corroborate the phenomenon that emergence and spread of atovaquone-resistant *P. falciparum* is very limited in the field.

INDONESIA