

Phenotypic analysis of atovaquone resistant Plasmodium berghei mutants

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Dr. Siregar is a researcher from Laboratorium Mitochondria and Infectious Diseases, Eijkman Institute for Molecular Biology, Jakarta, Indonesia.

Josephine has interest in the molecular biology of the energy transducing membrane of the malaria parasite in relation to antimalarial drug. She has produced most significant results in the understanding of the mechanism of action of Atovaquone and in the cytoplasmic genetic of the malaria parasite. Her work provides the first direct demonstration of the relationship between mutations in the mitochondrial cytochrome b gene and the resistance of respiratory complex III to atovaquone and reported a block of transmission of atovaquone resistance genotypes to newhosts.

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Introduction

Approximately, 40% of the world's populations, mostly those living in the world's poorest countries, are at risk of malaria. Every year, more than 500 million people become severely ill with malaria [1]. The expansion of drug-resistant strains of *P. falciparum* has caused serious global health problems in malaria treatment. Several new drugs have been introduced including new derivatives of quinoline-based compounds, but resistance of the parasite to those drugs developed very rapidly. Current understanding in the development of drug resistance is limited, although this knowledge could be used to (i) develop strategies to prevent or delay the development of drug resistance, and (ii) to modify the structure of drugs such that therapeutic function is maintained whereas drug resistance is minimized.

Atovaquone (a hydroxy-1,4-naphthoquinone), that shares structural similarity with ubiquinone, is a potent and specific inhibitor of the cytochrome bc1 complex, and its molecular target is known to be the ubiquinol oxidation pocket (Qo site) of the cytochrome bc1 complex [2-4]. It is effective against chloroquine-resistant strains of *Plasmodium falciparum* and is a major component of Malarone® which has been widely used in endemic regions in Africa and Thailand. Mutations in the mitochondrial DNA (mtDNA) cytochrome b gene were shown to associate with resistance to atovaquone in various organisms, such as *Plasmodium* species, *Toxoplasma*, *Pneumocystis carinii* and yeast [5-10]. In the *Plasmodium* spp., 10 mutations, M133I, L144S, I258M, F267I, Y268C/N/S, L271F/V, K272R, P275T, G280D and V284F had been documented in the quinone binding domains 1 (Qo1) and 2 (Qo2) of the cytochrome b protein.

The atovaquone-resistance mutations in the mitochondrial DNA encoding the *Cyt b* gene of *Plasmodium berghei* affect the growth of the blood stages of the parasite and their development in mosquitoes [10, 11]. The mutations in the *Cyt b* gene then appear to have resulted in a reduced functioning of the cytochrome bc1 complex, thereby causing the electron transport-chain to become less efficient [12]. The defect in this enzyme function may lead to the phenotype change of *P. berghei* atovaquone resistant mutants. In this study, we investigated the phenotype change caused by atovaquone resistance in *P. berghei* by analysis of the over-expression profile of the *Cyt b* gene in the different mutations.

Materials and Methods

P. berghei atovaquone-mutant isolates that were generated in our laboratory as previously reported [5, 10], were used in this study. Total RNA was isolated from erythrocytes of mice infected with *P. berghei* parasites using standard procedures, as described before [13]. The isolated RNA was cleaned from contaminating genomic DNA by DNase I treatment. Subsequently, the RNA was used to amplify genes of interest by reverse transcriptase PCR using ImProm-II™ Reverse Transcriptase (Promega). Conventional PCR amplification was used to test all pair of primers and the products were analyzed on classical agarose gels to check the specificity of the DNA amplification for each gene of interest. Quantitative PCR techniques were performed in analysing the over-expression profile of the *cyt b* gene compared to *P. berghei*

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ANKA hypothetical protein gene (PB100384.00.0) in the different mutants.

Results and Discussion

cDNA of *P. berghei* atovaquone mutants and wild type was collected and the purity of all cDNA samples was good since the OD 260/280 ratio ranged from 1.78 – 1.84. All cDNA samples were used to examine the *cyt b* gene expression by quantitative PCR in quadrupole. A consistent pattern of *cyt b* gene expression was observed for 5 mutants (Y268C, Y268N, Y268S, M133I, and M133I/L271V) compared to wild type, except for mutant L271V/K272R (see figure 1.) Upregulation of *cyt b* expression in 5 *P. berghei* mutants compared to control varied from 1.7 to 2.4 fold. The highest increase in expression of the cytochrome *b* gene (2.4 fold) was shown in mutant parasites with the single mutation M133I located in quinone binding 1 (Qo1). The lowest increase was shown in mutant parasites with the double mutation L271V/K272R at quinone binding 2 (Qo2). The *cyt b* gene expression in the other mutant parasites, with single mutations located in quinone binding 2 (Qo2) Y268C, Y268S, Y268N and the double mutation M133I/L271V in quinone binding 1 (Qo1) and 2 (Qo2) were shown to be upregulated almost 2 times compared to the wild type. The distinct mutants are a sort of control for each other, as all mutants are affected in their enzymatic activity, and therefore, the cellular expression pattern response is expected to be the same.

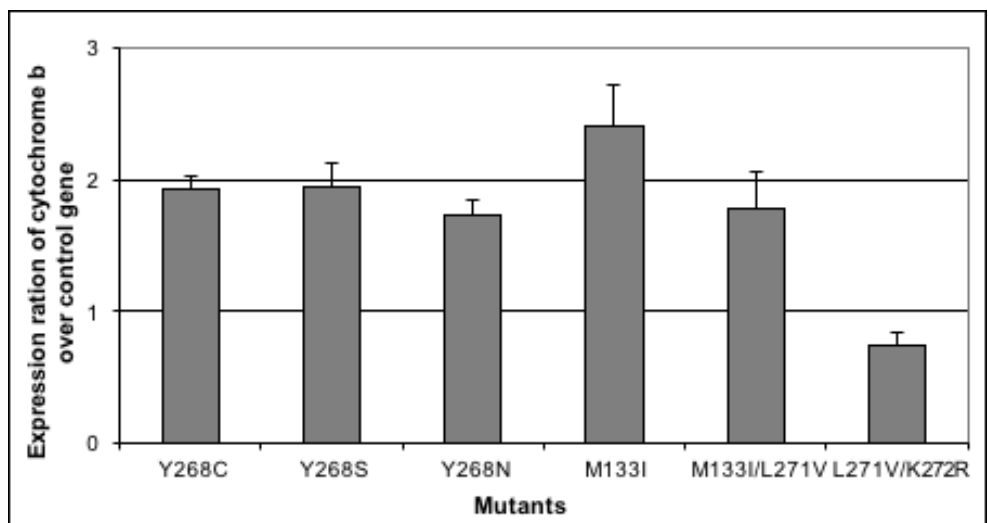


Figure 1. Expression *P. berghei* Cytochrome *b* mutants (normalized to control). Error bars represent the standard deviation (n=4).

Conclusions

The *cyt b* gene in the different mutants is over expressed compared to the wild type isolates. We need to repeat experiments to determine the exact expression ratio of *cyt b* over the control gene in the wild type, in order to estimate the natural range of variation among the wild type in expression ratio of *cyt b* over the mutant. In addition, we can use other mitochondrial genes to compare the expression of the *cyt b* gene.

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