

A preliminary study on possible existence of cytochrome P450 (CYP) gene in *Plasmodium falciparum*

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Abstract

The existence of cytochrome P450 (CYP) enzymes in *Plasmodium falciparum* as well as other protozoa may imply the role/function of CYP in parasite survival. Successful hybridization of CYP-specific probes (CYP 2B1/B2) to *P. falciparum* mRNA extracted from sensitive strains was reported. This evidence supported the existence of CYP genes in *P. falciparum* malaria. In the present study, we characterized CYP gene in *P. falciparum* and correlated CYP activities to antimalarial sensitivity *in vitro*. At the first step, an array of bioinformatic technique was used to analyze all currently sequenced protozoan genomes ([http://www.sanger.ac.uk/ Projects/ Protozoa/](http://www.sanger.ac.uk/Projects/Protozoa/)) including *Plasmodium spp.*, and with *P. falciparum* in particular. The initial BLAST sequences with the conserved CYP gene were performed, followed by more specific BLAST searches for sequences possessing CYP homology of human and protozoan database. Results from BLAST analyses showed no sequence alignments which would ascertain conserved nucleotide regions to be CYP gene in *P. falciparum*. This contradictory finding confirmed the previous report. Phenobarbital was added at 1 μ M concentration to the *in vitro* culture of early trophozoite stage *P. falciparum* for both chloroquine-sensitive (3D7) and -resistant (K1) clones and the culture was incubated at 37° C for 12 hr. Total RNA was isolated from the parasites and dot blot hybridization was performed using a rat cDNA probe covering exon 6-9 of the phenobarbital inducible CYP 2B1/B2 gene. The negative result was shown in this experiment. Based on this result together with the bioinformatics analyses, we suggested the absence of CYP 2B1/B2 gene in *P. falciparum* genome.

Keywords: *Plasmodium falciparum*, cytochrome P450, bioinformatics, hybridization

Introduction

CYP belongs to a superfamily of proteins ubiquitously found across the phylogenetic tree and are known to catalyze a multitude of enzymatic reaction types. There have been a number of reports in the literature indicating the existence of enzyme activity in *P. falciparum* that is attributable to cytochrome P450 (CYP) enzymes. The CYP-dependent activity was shown to be enhanced in *P. falciparum* resistant strains, and could also be induced by known classical CYP inducers (1-2). Successful hybridization of CYP-specific probes (CYP 2B1/B2) to *P. falciparum* mRNA extracted from sensitive strains, and with an increased level of binding in resistant strains, lends support to the existence of CYP-related genes in this malarial parasite (1). The possible existence of CYP or CYP-like enzymes in *P. falciparum* as well as that shown in other protozoa may imply the role/function of CYP in parasite survival (3).

In the present study, we characterized CYP or CYP-like gene in *P. falciparum* and investigated the association between CYP activity in *P. falciparum* to sensitivity of the parasite to antimalarial drugs *in vitro*. We used array of bioinformatic techniques to analyze all currently sequenced protozoan genomes ([http://www.sanger.ac.uk/ Projects/Protozoa/](http://www.sanger.ac.uk/Projects/Protozoa/)) including *Plasmodium spp.*, and with *P. falciparum* in particular. The initial blast sequences with the conserved CYP gene were performed, followed by more specific blast searches for

sequences possessing CYP homology of human and protozoan database. Results from blast analyses showed no sequence alignments which would ascertain conserved nucleotide regions of CYP gene in *P.falciparum*. Due this contradictory observation, we carried out the experiment to confirm the previous finding by Surolia et al. for the existence of CYP (CYP 2B1/B2) genes in *P.falciparum* malaria (1).

Method

Male rats of Sprague-Dawley strain (90-100 g) were given single injections of phenobarbital (8 mg/100 g, intraperitoneally). Rats were sacrificed after 24 hr and livers were collected for total RNA extraction by RNeasy Mini kit (QIAGEN). cDNA was prepared by using Omniscript Reverse Transcription (QIAGEN), and used as a template in polymerase chain reaction (PCR) for exon 6-9 of CYP 2B1/B2 gene. The forward and reverse primers used were as follows:

Forward primer: GATTCAGGAGGAAGCCCAAT

Reverse primer: TTTTCCAATGCCACTCTCC

PCR reactions were carried out in 25 µl volumes comprising 2 µl template, 1.0 µM primers, 1.0 mM magnesium chloride, 200 µM dNTP, 1X PCR Buffer and 0.1U of Taq DNA polymerase. PCR annealing cycles consisted of an initial four minutes denaturation step at 95°C, followed by 35 cycles of denaturation at 95°C for one minute, annealing at 55.5°C for one minute and extension at 72°C for one minute. Final extension was at 72°C for five minutes. The PCR amplifications were performed in a Bio-Rad thermal cycler. CYP 2B1/B2 PCR product size was 1,004 basepairs resolved by electrophoresis on 1.0% agarose gel and visualized by UV transillumination. PCR product was extracted from agarose gel using QIA quick spin kit (QIAGEN), then sequenced and aligned to exon 6-9 of rat CYP 2B1/B2 gene. PCR DIG labeling probe for rat cDNA CYP 2B1/B2 gene was prepared after compatible alignment of CYP 2B1/B2 was observed.

P.falciparum, chloroquine-sensitive (3D7) and chloroquine-resistant (K1) clones were cultured continuously *in vitro* by a modification of the methods of Trager and Jensen (4). Synchronization was done when 5% parasitemia was achieved to obtain only ring stage parasite. Phenobarbital (CYP-inducer) was added at 1.0 µM concentration to the culture at early trophozoite stage and incubation carried out for 12 hr (1). Total RNA was isolated from washed parasite pellet by using TRIZOL[®] reagent (Invitrogen). Dot blot analysis was carried out using standard procedure (5) with 10 and 25 µg of total RNA, respectively. A rat cDNA probe covering exon 6 to 9 of the phenobarbitone inducible CYP2B1/B2 gene was used (1). Hybridization was carried out in 50% formamide at 42°C and final washing included 0.2x SSC in 0.1% SDS at 65°C for 15 min (6).

Results

Total RNA of Sprague Dawley rat with good quality especially 28S and 18S band was obtained, with a huge PCR product of CYP 2B1/B2 (product size 1004 bps.) before extraction and sequencing it from gel. After sequencing, the alignment result showed that PCR product was the CYP 2B1/B2 fragment. CYP 2B1/B2 DIG labeling probe resulted in a little bigger size than its fragment which was none labeling. Based on the results of blot hybridization of CYP 2B1/B2 DIG labeling probe on total RNA of *P.falciparum* (K1 and 3D7 clones) in both control and 1 µM Phenobarbital, a negative result of CYP 2B1/B2 probe hybridization was shown with total RNA from both *P.falciparum* clones, whereas a positive result was obtained with total RNA from rat liver treated with phenobarbital.

Discussion

Results shown in the present study contradict with that reported previously by Surolia et al (1). The negative hybridization of CYP 2B1/B2 DIG labeling probe observed in total

RNA of *P.falciparum* in all groups suggests the absence of CYP 2B1/B2 gene in *P.falciparum*. The positive results previously reported could be explained by contamination of rat RNA with *P.falciparum* RNA. It was noted however that even with the absence of CYP-gene, metabolic activity was found in *Plasmodium* spp. (7). It is possible that other protein that acts like CYP enzyme (CYP-like protein) may contribute to this metabolic activity. Proteomics of the *P.falciparum* CYP-like protein is being investigated and correlate its activity with sensitivity of the parasites to antimalarial drugs.

Conclusion

Based on our results from bioinformatic analysis of *P.falciparum* genomes, together with that from hybridization experiment using CYP-specific probes (CYP 2B1/B2) from mRNA obtained from rat liver, with mRNA obtained from *P.falciparum* clones, we conclude for the absence of CYP gene sequences in *P.falciparum* genome.

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