Received: 19 June 2020 | Revised: 2 September 2020 | Accepted: 15 September 2020

DOI: 10.1111/bcp.14574

ORIGINAL ARTICLE

Impact of *Plasmodium vivax* malaria and antimalarial treatment on cytochrome P450 activity in Brazilian patients

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Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico; Departamento de Ciência e Tecnologia, Ministério da Saúde (DECIT),; Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro; Fundação de Amparo à Pesquisa do Estado de São Paulo; Fundação de Amparo à Pesquisa do Estado do Amazonas

Aims: To investigate the impact of *Plasmodium vivax* malaria and chloroquine-primaquine chemotherapy on CYP2D6 and CYP2C19 activity in patients from the Brazilian Amazon.

Methods: Adult patients (n = 30) were given subtherapeutic doses of CYP2D6 and CYP2C19 phenotypic probes metoprolol (10 mg) and omeprazole (2 mg) in three different stages of *vivax* malaria illness: acute disease (study phase 1), post chemotherapy (phase 2) and convalescence (stage 3). Plasma concentrations of probes and CYP-hydroxylated metabolites (α-OH metoprolol and 5-OH omeprazole) were measured using LC/MS/MS. Two pharmacokinetic metrics were used to estimate CYP activity: (a) ratio of plasma concentrations of probe/metabolite at 240 minutes after administration of the probes and (b) ratio of areas under the time-concentration curves for probe/metabolite ($AUC_{0\text{-}12h}$). For statistical analysis, the pharmacokinetic metrics were normalized to the respective values in phase 3. *Taqman* assays were used for CYP2D6 and CYP2C19 genotyping. Cytokines levels were measured using cytometric bead array.

Results: Both pharmacokinetic metrics for metoprolol and omeprazole, and plasma concentrations of cytokines IL-6, IL-8 and IL-10 varied significantly across the three study phases (ANOVA $P < 0.0001$). Post hoc tests showed greater metoprolol:α-OH metoprolol ratios in phases 1 and 2 compared to phase 3, larger omeprazole:5-OH omeprazole ratios in phase 1 than in phases 2 and 3, and higher circulating IL-6, IL-8 and IL-10 in phase 1 than in phases 2 and 3.

Conclusion: *P. vivax* malaria and treatment altered CYP2D6 and CYP2C19 metabolic phenotypes. CYP2C19 inhibition is attributed to a higher level of circulating proinflammatory cytokines, while suppression of CYP2D6 is ascribed mainly to chloroquine exposure.

The authors confirm that the Principal Investigator for this paper is Wuelton Marcelo Monteiro and that he had direct clinical responsibility for patients.

KEYWORDS
Brazilian Amazon, chloroquine, CYP2C19, CYP2D6, cytokines, *Plasmodium vivax* malaria, primaquine

1 | INTRODUCTION

Inflammation and infection have long been known to modulate pharmacokinetic processes, with potential therapeutic implications. Both phase I and phase II drug-metabolizing enzymes and drug transporters are targeted by the inflammatory response through mechanisms triggered by increased circulating levels of proinflammatory cytokines, such as IL-1β, IL-6, tumour necrosis factor alpha (TNFα), and interferons (IFN).[1–5] Cytochrome-P450 enzymes (CYPs) are major targets of acute and chronic inflammatory conditions: expression and activity of these important drug-metabolizing enzymes are reduced in clinical settings of high concentrations of circulating proinflammatory cytokines, such as cancer and congestive heart failure.[5–8]

Increased levels of circulating proinflammatory cytokines are hallmarks of human parasitic diseases such as leishmaniasis[9,10] and malaria.[11–15] We have previously shown that the phenotypic activities of CYP3A4 and CYP2C19 were significantly reduced during acute visceral leishmaniasis and restored after curative chemotherapy; suppression of CYP activity was attributed to increased plasma concentrations of proinflammatory cytokines during active disease.[16]

Regarding human malaria, there is only limited information for *Plasmodium falciparum*, suggestive of reduced activity of CYP1A2 and CYP3A enzymes.[17–20] However, four other *Plasmodium* sp. are malaria pathogens worldwide, among these is *P. vivax*, the most geographically widespread human malaria parasite, which prevails in the Brazilian Amazon, where the present study was conducted. We investigated the phenotypic activity of **CYP2D6** and CYP2C19 in the course of acute *vivax* malaria, after completion of antiparasite chemotherapy with **primaquine**/**chloroquine** and during convalescence. CYP selection was guided by the evidence that CYP2D6 is the major pathway for conversion of primaquine into its active metabolite(s)[21–27] and by our previous findings of reduced CYP2C19 activity during the acute phase of visceral leishmaniosis.[16]

What is already known about this subject

- Inflammation and infection are known to modulate pharmacokinetic processes, with potential therapeutic implications. Cytochrome P450 (CYP) enzymes are major targets of inflammatory conditions: expression and activity of these important drug metabolizing pathways are reduced in clinical settings of high concentrations of circulating proinflammatory cytokines. Increased levels of proinflammatory and regulatory cytokines are hallmarks of human parasitic diseases, such as leishmaniasis and malaria. Suppression of CYP3A and CYP2C19 in acute visceral leishmaniasis has been reported and there is limited evidence of reduced activity of CYP1A2 and CYP3A in *P. falciparum* malaria. However, the impact of *P. vivax*, the most geographically widespread human malaria parasite, on CYP enzymes is largely unknown.

What this study adds

- This is the first study to investigate the impact of *vivax* malaria and antimalarial treatment on the activity of two major drug-metabolizing enzymes, namely CYP2D6 and CYP2C19. A remarkable strength of this study is that it reflects real-life management of *vivax* malaria patients in an endemic area of the Brazilian Amazon. Pharmacokinetic metrics obtained with the validated phenotypic probes metoprolol (CYP2D6) and omeprazole (CYP2C19) revealed that both CYPs were inhibited during acute disease; after 7 days of combined chloroquine-primaquine chemotherapy CYP2C19 activity was restored, whereas CYP2D6 remained suppressed. CYP2C19 inhibition is attributed to increased plasma concentrations of proinflammatory cytokines, whereas exposure to chloroquine is thought to be the major culprit for suppression of CYP2D6 activity.

2 | METHODS

The study procedures were approved by the local Human Research Ethics Committee of the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD) (approval number 1.126.290/2015). All participants provided written informed consent.

2.1 | Study population

We enrolled 30 unrelated adults (22 men), aged 23-59 years old, self-identified according to the Brazilian Census as Brown (*Pardo* in Brazilian Portuguese, n = 26), White (n = 2) or Amerindian (n = 2), presenting with an acute episode of nonsevere *P. vivax* malaria at the FMT-HVD in Manaus, Amazonas State, Brazil. All patients were treated with the therapeutic scheme recommended by the Brazilian Ministry of Health for *P. vivax* uncomplicated malaria in adults, which consists of combination of oral chloroquine (150 mg/day, 3 days) and

primaquine (30 mg/day, 7 days). Drug treatment was supplied free of cost to all patients through the Brazilian Public Health System.[28]

2.2 | Study design

This open-label study comprised three phases: Phase 1 within 24 hours of diagnosis of the acute malaria episode, phase 2 within 24 hours after completion of the 7-day antimalarial chemotherapy and phase 3 during convalescence, 5-6 weeks after chemotherapy. In each phase, following an overnight (>8 hours) fast, the patients were admitted to a clinical research ward at the FMT-HVD, a catheter was introduced in a superficial vein, and a baseline (predosing) blood sample (4 mL) was collected. Each patient then ingested a capsule containing subtherapeutic doses of **metoprolol** (10 mg) and **omeprazole** (2 mg), used as phenotypic probes for CYP2D6 and CYP2C19 activity, respectively.[29] Blood samples were drawn at 90, 120 and 240 minutes after ingestion of metoprolol and omeprazole. A standard breakfast was provided after collection of the 120-minute sample. The patients remained under observation by a physician and a protocol nurse until collection of the 240-minute blood sample and were subsequently discharged. The patients were instructed to return to the research ward at FMT-HVD in case of malaria symptoms throughout the study. In phases 2 and 3, thick blood smears were collected for detection of asymptomatic cases; patients diagnosed with malaria recurrence at these phases were excluded from data analyses.

2.3 | Laboratory procedures

Malaria diagnosis was performed by light microscopy by an experienced microscopist.[30] Afterwards, real-time PCR was performed to confirm *P. vivax* mono-infection. The extraction of total DNA from whole blood was performed using a QIAamp DNA Blood Mini Kit (Qiagen Inc, Germantown, MD, USA), according to the manufacturer's protocol. The DNA was amplified in an Applied Biosystems 7500 Fast System using primers and TaqMan fluorescence labelled probes for real-time PCR.[31]

A 200-μL aliquot of the predosing blood sample from phase 1 was used for DNA extraction and subsequent genotyping of commonly reported *CYP2C19* and *CYP2D6* Single Nucleotide Polymorphism (SNPs) (Table S1), *CYP2D6* gene deletion and multiplication, using Taqman allele discrimination assays. The remaining volume of the predosing phase 1 sample and all other blood samples were centrifuged at room temperature within 15 minutes after collection, and the plasma was separated and stored at −20 °C. Validated LC/MS/MS methods[32] were used for quantification of plasma concentrations of metoprolol, omeprazole and their CYP-hydroxylated metabolites α-OH metoprolol and 5-OH omeprazole, respectively. Plasma concentrations of the cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12p70 (IL-12p70) and tumour necrosis factor (TNF) were performed by the cytometric bead array (CBA) technique, with BD CBA Human Inflammatory Cytokine (BD Biosciences, San Diego, CA, USA). A FACSCanto II flow cytometer was used for sample acquisition. The analysis of cytokine concentrations was conducted on FCAP-Array software v.3 (BD Biosciences).

2.4 | Estimation of CYP2D6 and CYP2C19 metabolic activity

Two metrics were adopted to estimate CYP metabolic activity: the first was based on the ratio of plasma concentrations of probe/metabolite at 240 minutes after administration of metoprolol and omeprazole.[33–35] The second metric applied a limited sampling strategy (LSS) validated for subtherapeutic doses of omeprazole and metoprolol in Brazilians.[36] Briefly, the ratio of plasma concentrations of probe:metabolite at 90, 120 and 240 minutes were inserted into LSS linear regression equations to estimate the ratio of the area under the time-concentration curves ($AUC_{0\text{-}12h}$) for metoprolol/α-OH metoprolol and omeprazole/5-OH omeprazole.[36]

2.5 | Estimation of metoprolol and omeprazole systemic exposure

The area under the plasma concentration versus time curve between 90 and 240 minutes ($AUC_{90\text{-}240min}$) obtained by the trapezoidal method was used to estimate the systemic exposure to metoprolol and omeprazole.

2.6 | Definition of CYP2D6 and CYP2C19 alleles, diplotypes and phenotypes

CYP2D6 and *CYP2C19* haplotypes were defined according to the star allele (*) nomenclature adopted by the Pharmacogene Variation (*PharmVar*) Consortium (https://www.pharmvar.org) based on the interrogated *CYP2D6* and *CYP2C19* SNPs, and *CYP2D6* copy number. The wild-type (*1) allele was attributed to each gene by default. *CYP2D6* haplotypes and diplotypes were inferred using HaploStats software (version 1.7.7) implemented on the R platform. The activity score (AS) system[37] was used to define the perceived functionality of the *CYP2D6* diplotypes and to assign four CYP2D6 phenotypes: normal metabolizer (NM), intermediate metabolizer (IM), poor metabolizer (PM) and ultrarapid metabolizer (UM). CYP2C19 phenotypes (NM, IM and UM) were assigned according to the CPIC guidelines[38] based on the SNPs interrogated (Supporting Information Table S1).

2.7 | Statistical analyses

Pharmacokinetic metrics and plasma concentrations of cytokines are presented as means (95% confidence interval, 95%CI). Statistical analyses of the CYP pharmacokinetic metrics were performed after

normalization of the individual values in phases 1 and 2 to the respective values in phase 3 (convalescence), which were taken as 1.0. Statistical analyses of cytokine data were based on the measured plasma concentrations. Differences across the three study phases were assessed by analysis of variance (ANOVA). When a significant difference was detected by ANOVA, post hoc pairwise comparisons between phases were carried out using the *t*-test with Tukey's correction. Values of $P < 0.05$ were considered to indicate statistically significant differences.

2.8 | Sample size determination

Based on previous data for healthy Brazilians exposed to the same subtherapeutic doses of metoprolol and omeprazole,[32] we calculated that 22 patients were required for having 80% power (β = 0.2) to detect at the 5% significance level (α = 0.05), a 25% difference in the CYP2D6 and CYP2C19 metabolic ratios between phases 1 (acute malaria episode) and 3 (convalescence). Considering the social-economic status of the patients presenting with acute malaria at our institution, the fact that they may reside at a considerable distance from the study centre and, additionally, that they might be at risk of reinfection before completing phase 3 of the clinical protocol, 30 patients were enrolled in the study.

2.9 | Nomenclature of targets and ligands

Key protein targets and ligands are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

3 | RESULTS

Three patients failed to complete the study protocol and three others had recurrence of *vivax* malaria between phases 2 and 3. These six patients were excluded from the data analyses, which were applied to the remaining 24 patients. There were no adverse events related to the CYP probe drugs that required medical intervention. We describe the findings for CYP2D6 and CYP2C19 separately.

3.1 | CYP2D6

The individual *CYP2D6* genotypes and assigned metabolizer phenotypes are presented in Supporting Information Table S2. Figure 1A shows the distribution of the metoprolol phenotypic metrics according to the CYP2D6 phenotypes. The phenotypic data refer to study phase 3 (convalescence) and are taken as representing the baseline CYP2D6 activity. The individual values of the two metoprolol pharmacokinetic metrics were highly correlated (R^2 = 0.98) and showed considerable overlap across CYP2D6 NM (n = 14), IM (n = 6)

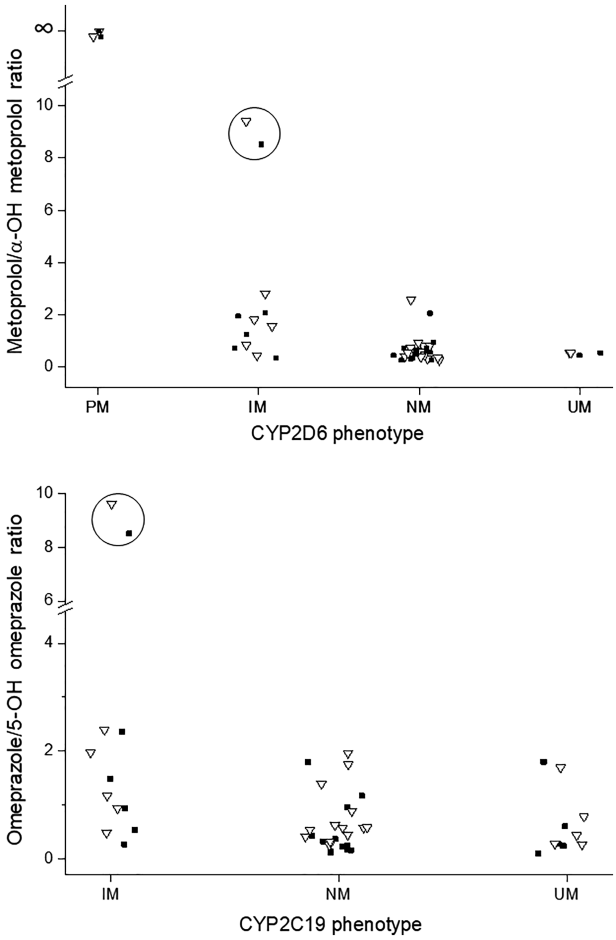

FIGURE 1 Distribution of metoprolol (A) and omeprazole (B) pharmacokinetic metrics according to the respective CYP2D6 and CYP2C19 metabolizer phenotypes. Data refer to phase 3 of the study (convalescence from acute *vivax* malaria episode). Open triangles, ratio of probe/metabolite concentrations in plasma 4 hours after oral administration of the probes metoprolol (10 mg) and omeprazole (2 mg). Black squares, ratio of the areas under the plasma concentration versus time curves ($AUC_{0\text{-}12h}$) of probe/metabolite, estimated by limited sampling regression equations. Metabolites: α-OH metoprolol and 5-OH omeprazole. PM, poor metabolizer; IM, intermediate metabolizer; NM, normal metabolizer; UM, ultra-rapid metabolizer. Circles denote outliers

and UM (n = 2). Of note, among IM patients, there was a distinct outlier (data circled in Figure 1A). This patient (number 13 in Supporting Information Table S2) was genotyped as *CYP2D6*41/*4*, with attributed activity score = 0.5, whereas all other IM patients had activity scores of 1.0 (Supporting Information Table S2). Two patients, classified as PMs (genotypes **4/*4* and **4/*4x2*), had undetectable α-OH metoprolol in plasma (hence the infinite ratios in Figure 1A), and were excluded from all subsequent statistical analyses.

The individual CYP2D6 metabolic ratios are plotted in Figure 2 and summarized in Table 1. Both metoprolol metrics differed significantly across the three study phases (ANOVA $P < 0.0001$). Post hoc Tukey tests showed significantly greater CYP2D6 metabolic ratios in phases 1 and 2 compared to phase 3, but no difference between

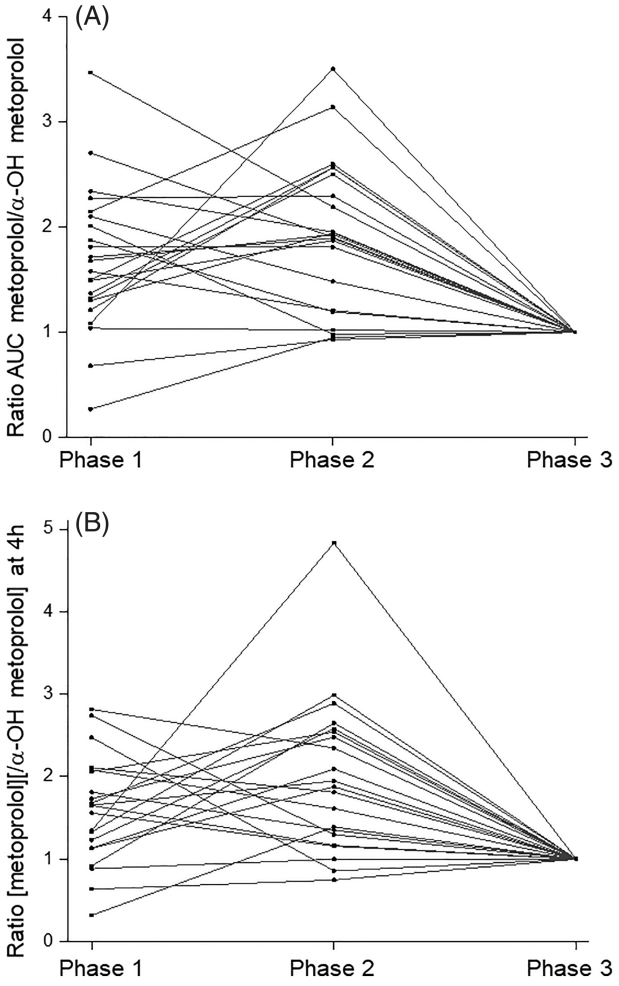

FIGURE 2 Individual values of the metoprolol pharmacokinetic metrics in the three study phases: phase 1, acute *vivax* malaria; phase 2, after completion of the combined chloroquine-primaquine treatment; phase 3, convalescence. Data normalized to the values measured in phase 3. Pharmacokinetic metrics: A, ratio of the areas under the plasma concentration versus time curves ($AUC_{0\text{-}12h}$) of metoprolol/α-OH metoprolol, estimated by limited sampling regression equations; B, metoprolol/α-OH metoprolol concentration ratio in plasma 4 hours after oral administration of 10 mg of metoprolol,

phases 1 and 2 (Table 1). Compared to phase 3, the CYP2D6 metabolic ratios were, on average, 1.59-1.67-fold higher in acute disease (phase 1) and 1.93-1.98-fold higher after completion of the 7-day malaria chemotherapy (phase 2).

We plot in Figure 3A the individual values of metoprolol $AUC_{90\text{-}240min}$ and the data are summarized in Table 2. On average, the $AUC_{90\text{-}240min}$ values in phases 1 and 2 were 1.35 and 1.63-fold greater than in phase 3, respectively. ANOVA confirmed a significant difference across the three study phases ($P < 0.0001$), and post hoc Tukey tests disclosed significant higher $AUC_{90\text{-}240min}$ in phases 1 and 2 compared to phase 3, but no difference between phases 1 and 2.

3.2 | CYP2C19

The distribution of omeprazole phenotypic metrics according to the CYP2C19 metabolizer phenotypes (Supporting Information Table S2) is plotted in Figure 1B. The individual values of the omeprazole metrics were strongly correlated (R^2 = 0.97) and overlapped across CYP2D6 NM (n = 13), IM (n = 5) and UM (n = 6). The IM phenotype was assigned to patients genotyped as *CYP2C19*1/*2* (n = 5) or *CYP2C19*1/*3* (n = 1). The patient with the *CYP2C19*1/*3* genotype (patient 1 in Supporting Information Table S2) showed considerable higher values for both omeprazole phenotypic metrics (data circled in Figure 1B) relative to the other IM individuals.

The individual CYP2C19 metabolic ratios are plotted in Figure 4 and summarized in Table 1. Both omeprazole metrics differed significantly across the three study phases (ANOVA $P < 0.0001$). Post hoc Tukey tests showed significantly greater CYP2C19 metabolic ratios in phase 1 compared to phases 2 and 3, and no difference between phases 2 and 3 (Table 1).

The omeprazole $AUC_{90\text{-}240min}$ (Figure 3B and Table 2) varied significantly across the study phases (ANOVA $P < 0.0001$), being, on average, 3-fold greater in phase 1 compared to phases 2 and 3. ANOVA indicated significant variation in omeprazole $AUC_{90\text{-}240min}$ across the three phases ($P < 0.0001$) and post hoc Tukey tests confirmed significant larger $AUC_{90\text{-}240min}$ in phase 1 compared to phases 2 or 3, and no difference between phases 2 and 3.

TABLE 1 Metoprolol and omeprazole metabolic ratios in *P. vivax* malaria patients

		Metabolic ratio*					Tukey *t*-test***	
CYP probe	***n***	**Phase 1**	**Phase 2**	**Phase 3**	**ANOVA****	**1 *vs* 2**	**1 *vs* 3**	**2 *vs* 3**
Metoprolol								
4 h ratio [metoprolol]/[α-OH metoprolol]	22	1.59 (1.33-1.85)	1.98 (1.60-2.36)	1	**<0.0001**	0.14	**0.03**	**<0.0001**
AUC ratio metoprolol/α-OH metoprolol	22	1.67 (1.39-1.95)	1.93 (1.64-2.22)	1	**<0.0001**	0.52	**0.001**	**<0.0001**
Omeprazole								
4 h ratio [omeprazole/5-OH omeprazole]	24	1.70 (1.32-2.08)	0.92 (0.72-1.12)	1	**<0.0001**	**<0.0001**	**0.001**	0.84
AUC ratio omeprazole/5-OH omeprazole	24	1.58 (1.17-1.99)	0.89 (0.53-1.25)	1	**<0.0001**	**<0.0001**	**0.01**	0.75

*Ratio of plasma concentrations of probe/metabolite, normalized to the respective invididual values in phase 3, expressed as mean (CI95%).
**P values for ANOVA; significant values shown in bold.
***P values for post hoc Tukey t-tests of pairwise comparisons between phases 1, 2 and 3; significant values shown in bold.

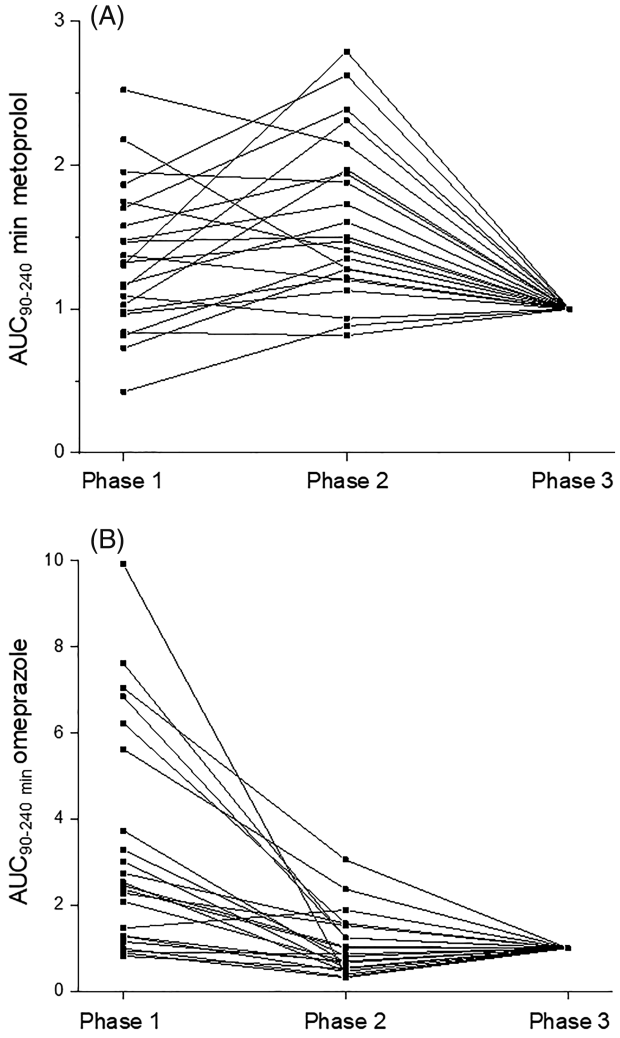

FIGURE 3 Effect of *vivax* malaria and antimalarial chemotherapy on systemic exposure to metoprolol and omeprazole. The plots show the individual areas under the plasma concentration versus time curves of metoprolol (A) and omeprazole (B) between 90 and 240 minutes ($AUC_{90\text{-}240min}$) after oral administration of 10 mg of metoprolol and 2 mg of omeprazole in the three study phases: phase 1, acute *vivax* malaria; phase 2, after completion of the combined chloroquine-primaquine treatment; phase 3, convalescence. Data normalized to the values measured in phase 3

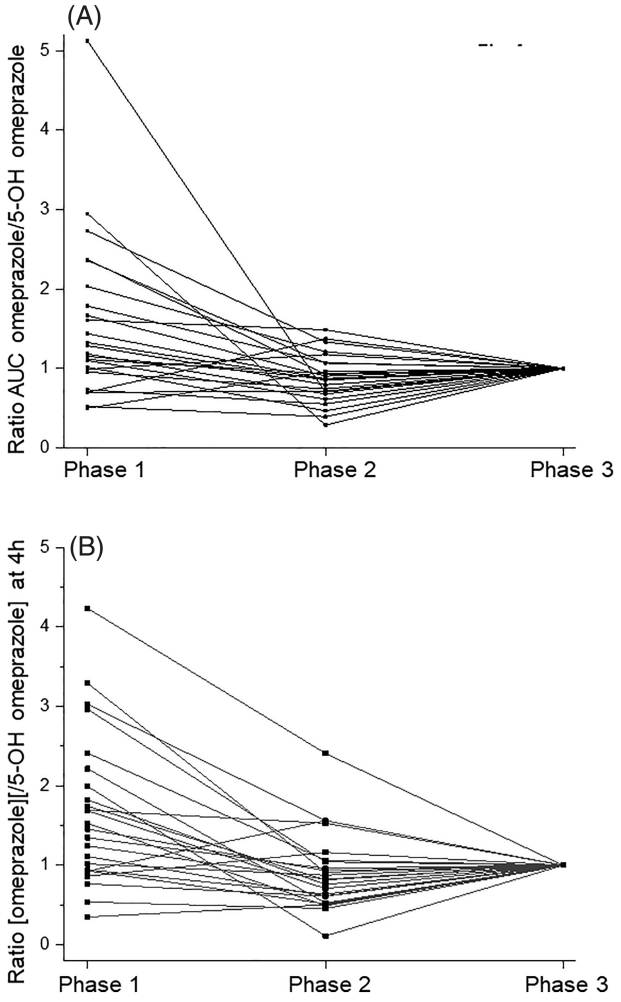

FIGURE 4 Individual values of the omeprazole pharmacokinetic metrics in the three study phases: phase 1, acute *vivax* malaria; phase 2, after completion of the combined chloroquine-primaquine treatment; phase 3, convalescence. Data normalized to the values measured in phase 3. Pharmacokinetic metrics: A, ratio of the areas under the plasma concentration versus time curves ($AUC_{0\text{-}12h}$) of omeprazole/5-OH omeprazole, estimated by limited sampling regression equations; B, omeprazole/5-OH omeprazole concentration ratio in plasma 4 hours after oral administration of 2 mg of omeprazole

TABLE 2 Metoprolol and omeprazole $AUC_{90\text{-}240min}$ in *P. vivax* malaria patients

		$AUC_{90\text{-}240min}$*					Tukey *t*-test***	
CYP probe	*n*	Phase 1	Phase 2	Phase 3	ANOVA**	1 *vs* 2	1 *vs* 3	2 *vs* 3
Metoprolol	22	1.35 (1.15-1.55)	1.63 (1.40-1.85)	1	**<0.0001**	0.09	**0.02**	**<0.0001**
Omeprazole	24	3.33 (1.15-4.36)	1.04 (0.76-1.32)	1	**<0.0001**	**<0.0001**	**<0.0001**	0.99

*$AUC_{90\text{-}120min}$, area under the plasma concentration versus time curve between 90 and 240 min, normalized to the respective individual values in phase 3. Data presented as mean (95%CI).

**P values for ANOVA; significant values shown in bold.

***P values for post hoc Tukey *t*-tests of pairwise comparisons between phases 1, 2 and 3; significant values shown in bold.

3.3 | Cytokine measurements

Plasma concentration data for the investigated cytokines are presented in Table 3. ANOVA detected no difference in IL-1b, TNF or IL-12-p70 concentrations across the three study phases, whereas significant differences were observed for IL-6, IL-8 and IL-10. Pairwise comparisons showed significantly higher concentrations of IL-6, IL-8 and IL-10 in acute *P. vivax* malaria (phase 1) relative to phases 2 (post-chemotherapy) and 3 (convalescence). For IL-6 and IL-10 there was no difference between phases 2 and 3, ie, the plasma concentrations of these cytokines were restored to baseline values after 7 days of antimalarial chemotherapy. IL-8 concentrations, however, remained elevated at the end of chemotherapy. Individual data for IL-6, IL-8 and IL-10 are plotted in Figure 5.

4 | DISCUSSION

This is the first study to investigate the impact of *vivax* malaria and antimalarial treatment on the activity of two major drug-metabolizing enzymes, namely CYP2D6 and CYP2C19. Pharmacokinetic metrics obtained with the validated phenotypic probes metoprolol (CYP2D6) and omeprazole (CYP2C19), revealed that both CYPs were inhibited during acute disease (phase 1); after 7 days of combined chloroquine-primaquine chemotherapy CYP2C19 activity was restored, whereas CYP2D6 remained suppressed. We suggest that distinct mechanisms may contribute to suppression of CYP activity and account for the different time courses of CYP2D6 and CYP2C19 inhibition.

Cytokine-induced inhibition of the expression and activity of CYP enzymes in hepatocytes is one likely mechanism. The host immune response against *P. vivax* triggers production and increased circulating levels of several proinflammatory and regulatory cytokines, which influence the pathogenesis and severity of the disease, and recurrence of acute episodes[11–15] Our results verified the increase in circulating IL-6, IL-8 and IL-10 in acute *vivax* malaria (phase 1), and reversal to baseline (IL-6 and IL-10) or considerable reduction (IL-8) at the end of the 7-day combined chloroquine-primaquine treatment (phase 2), in agreement with previous results.[11] The time course of changes in circulating IL-6 and IL-10, and to a lesser degree IL-8, parallels the transient inhibition of CYP2C19 activity observed in phase 1 of this study. Increased plasma concentrations of proinflammatory cytokines

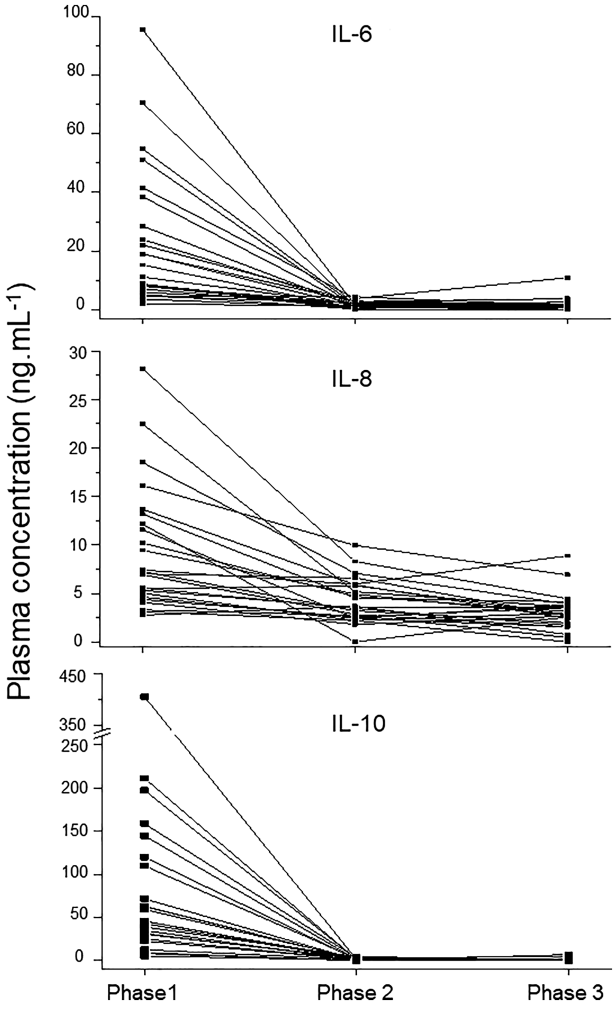

FIGURE 5 Individual plasma concentrations of cytokines IL-6 (A), IL-8 (B) and IL-10 (C) in the three study phases: phase 1, acute *vivax* malaria; phase 2, after completion of the combined chloroquine-primaquine treatment; phase 3, convalescence

TABLE 3 Plasma concentrations of cytokines in *P. vivax* malaria patients

	Plasma concentration of cytokines (pg.mL^{-1})*				Post hoc test***		
Cytokine	Phase 1	Phase 2	Phase 3	ANOVA**	1 *vs* 2	1 *vs* 3	2 *vs* 3
IL-1β	4.67 (0.35-9.00)	3.66 (0.61-6.72)	3.58 (0-8.35)	0.23	0.53	0.53	0.33
IL-6	23.26 (13.62-32.90)	1.61 (1.18-2.04)	1.65 (0.76-2.53)	**<0.0001**	**<0.0001**	**<0.0001**	0.92
IL-8	9.49 (6.88-12.10)	4.25 (3.34-5.17)	3.04 (2.28-3.80)	**<0.0001**	**<0.0001**	**<0.0001**	**0.006**
IL-10	81.52 (45.17-117.88)	1.61 (1.21-2.01)	1.36 (0.74-1.98)	**<0.0001**	**<0.0001**	**<0.0001**	0.52
IL-12p70	0.46 (0-0.91)	0.40 (0.02-0.79)	0.53 (0-1.20)	0.09	0.86	0.86	0.75
TNF	1.03 (0.05-2.01)	0.71 (0-1.58)	0.93 (0-1.86)	0.23	0.48	0.48	0.35

*Data expressed as mean (95%CI).

**P values for repeated measures ANOVA, significant values shown in bold.

***P values for pairwise comparisons (Tukey *t*-test) between phases 1, 2 and 3; significant values shown in bold.

have been implicated in the suppression of the metabolic activity of distinct CYP enzymes in multiple clinical settings,[2,8] including visceral leishmaniosis, another parasitic disease highly prevalent in Brazil.[16] We suggest that a similar mechanism, triggered by the increased circulating levels of the proinflammatory cytokines IL-6 and IL-8, accounts for the suppression of CYPC19 activity during the acute phase of *vivax* malaria. This suggestion is consistent with the reported inhibition of selected CYPs in murine malaria models, associated with proinflammatory cytokines.[39–42]

The regulatory cytokine IL-10, although detected at considerably higher concentrations in study phase 1, has not been linked to inhibition of CYP enzymes in other clinical or experimental settings of inflammation.[3,8] Indeed, daily administrations of IL-10 to healthy volunteers induced an acute-phase response, but did not alter CYP1A2, CYP2C9 and CYP2D6 activities, as measured by selective phenotypic probes.[43]

In contrast to CYP2C19, regulation of CYP2D6 activity in inflammation has not been consistently demonstrated.[3,5] The distinct time courses of increased circulating proinflammatory cytokines and changes in CYP2D6 pharmacokinetic metrics suggest that additional mechanism(s) account for suppression of CYP2D6 activity in acute *vivax* malaria and after completion of chloroquine-primaquine chemotherapy. There is a wealth of clinical and experimental data pointing to inhibitory effects of chloroquine on CYP2D6 activity. Lancaster et al[44] showed that chloroquine inhibited CYP2D6-mediated α-hydroxylation of metoprolol by human liver microsomes; importantly, this is the same metabolic reaction underlying our use of metoprolol as a CYP2D6 probe. Inhibition of CYP2D6 by chloroquine in human liver microsomes was confirmed by Masirimembwa et al[45] using bufuralol as substrate. The Ki values for CYP2D6 inhibition were within the range of chloroquine concentrations in liver following usual antimalarial doses, which prompts the suggestion that chloroquine is capable of decreasing CYP2D6 activity in vivo. Accordingly, Somooya et al[46] verified inhibition of CYP2D6-mediated hydroxylation of debrisoquine—another selective CYP2D6 phenotype probe—in healthy Zambians 2 hours and 1 week after chloroquine dosing, and reversal of the inhibition at 2 weeks. Of particular relevance to *vivax* malaria are the findings of the pharmacokinetic interactions between chloroquine and primaquine involving CYP2D6 activity. Pukrittayakamee et al[47] reported that chloroquine increased the plasma levels of primaquine and its major metabolite, carboxyprimaquine, in healthy subjects, whereas Fasinu et al[48] showed that chloroquine inhibited the CYP2D6-mediated clearance of primaquine in human hepatocytes, with very modest effects on the oxidative deamination products of monoamino oxidase, the other major metabolic pathway for primaquine metabolism in humans. The apparent discrepancy between the well-demonstrated enhancement of primaquine antimalarial efficacy by chloroquine and the compelling evidence that CYP2D6 activity is essential to the antimalarial efficacy of primaquine has been tentatively explained by the contribution of active primaquine metabolites that are not CYP2D6-dependent, and by transporter (ABCB1)-mediated primaquine-chloroquine interactions affecting the drugs' pharmacokinetics.[22,48] Human pharmacokinetic studies, comprising active primaquine metabolites, are warranted to fill the knowledge gaps regarding the effects of chloroquine suppression on CYP2D6 and its potential consequences on *vivax* malaria radical cure.

Collectively, the data presented in the preceding paragraph support the notion that chloroquine-induced inhibition of CYP2D6 activity provides a major mechanism for the increased metoprolol/α-OH metoprolol ratio and systemic exposure to metoprolol upon completion of the antimalarial treatment (phase 2). A similar mechanism may account, at least in part, for the increased metoprolol metabolic ratio and systemic exposure in phase 1, which was carried out after initiation of chloroquine-primaquine treatment, following diagnosis of the acute *vivax* episode. Inhibition of CYP26 by proinflammatory cytokines cannot be excluded as contributing to the changes in metoprolol pharmacokinetic metrics in phase 1, although, as mentioned above, regulation of CYP2D6 activity in inflammation has never been consistently demonstrated.[3,5] Furthermore, there was a small increase in the metoprolol pharmacokinetic metrics of CYP2D6 inhibition at the end of the chloroquine-primaquine treatment, whereas the plasma concentrations of proinflammatory cytokines IL-6 and IL-8 declined.

In conclusion, this is the first investigation of the impact of *vivax* malaria and curative chemotherapy on the activity of two major drug-metabolizing enzymes. We report that the phenotypic activities of CYP2D6 and CYP2C19, assessed by the selective probes metoprolol and omeprazole, were suppressed in the acute phase of *vivax* malaria and that chloroquine-primaquine treatment restored CYP2C19, but not CYP2D6, activity. CYP2C19 inhibition is attributed to increased plasma concentrations of proinflammatory cytokines, whereas exposure to chloroquine is thought to be the major culprit for suppression of CYP2D6 activity.

An important strength of this study is that it reflects real-life management of *vivax* malaria patients in an endemic area in the Brazilian Amazon. Nevertheless, we acknowledge limitations. First, the prescribed antimalarial treatment followed the recommendations of the Brazilian Ministry of Health for *P. vivax*, but drug administration was not supervised and the possibility of poor adherence cannot be excluded. In addition, we cannot exclude undisclosed use by patients of nonprescribed drugs or xenobiotics that might affect CYP activity or undetected subpatent (ie, submicroscopic) recurrent infections after treatment completion. Second, the metoprolol/α-OH-metoprolol and omeprazol/5-OH-omeprazol ratios were taken as measures for CYP activity. However, these ratios could also be affected by other factors during the acute disease phase, including differential changes between drug and metabolite in protein binding (acute phase proteins), other changes affecting volume of distribution, changes in liver perfusion, etc, which were not explored in this study. Finally, the results of this study derive from patients with uncomplicated *vivax* malaria and may not predict changes in CYP activity in the rarer, severe form of the disease.

ACKNOWLEDGEMENTS

This study was supported by the Brazilian agencies Conselho Nacional de Pesquisa e Desenvolvimento (CNPq), Departamento de Ciência e Tecnologia, Ministério da Saúde (DECIT), Fundação de Amparo à

Pesquisa do Amazonas (FAPEAM; PAPAC 005/2019 and Pró-Estado), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2018/05616-3).

CONTRIBUTORS

G.S.-K. designed the study protocol and wrote the original manuscript. G.C.M., W.M.M. and M.V.G.L. supervised the clinical and laboratory work in Manaus. A.C.G.A., E.F.G.F., F.R.S. and L.W.B. enrolled patients, managed laboratorial work in Manaus and performed the follow-up of the patients. A.B.R.E. and A.C.G.A. genotyped CYP polymorphisms. A.G.C. carried out the cytokine analyses. M.P.M. and V.L.L. measured the drug concentrations in plasma. G.S.-K., V.L.L. and A.C.G.A. analysed the data. All authors read and approved the final manuscript.

COMPETING INTERESTS

There are no competing interests to declare.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Almeida AC, Elias ABR, Marques MP, et al. Impact of *Plasmodium vivax* malaria and antimalarial treatment on cytochrome P450 activity in Brazilian patients. *Br J Clin Pharmacol*. 2021;87:1859–1868. https://doi.org/10.1111/bcp.14574