British Journal of Clinical Pharmacology

DOI:10.1111/bcp.12677

Impact of visceral leishmaniasis and curative chemotherapy on cytochrome P450 activity in Brazilian patients

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Keywords
Brazil, CYP2C19, CYP2C9, CYP3A4, IL-6, visceral leishmaniasis

Received
17 February 2015

Accepted
29 April 2015

Accepted Article Published Online
2 May 2015

WHAT IS ALREADY KNOWN ABOUT THE SUBJECT

- Visceral leishmaniasis (VL or kala-azar) has an estimated yearly incidence of 0.2 to 0.4 million cases worldwide, 90% of which occur in six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan.
- Leishmania infection leads to increased circulating levels of interleukin (IL) 6, IL-12, IL-17, interferon γ and tumour necrosis factor α during active disease.
- Expression and activity of CYP enzymes are affected in the setting of high concentrations of proinflammatory cytokines in cancer and congestive heart failure, but in human parasitic diseases there is only limited information for malaria.

WHAT THE STUDY ADDS

- The metabolic activity of CYP3A4 and CYP2C19, but not CYP2C9, was significantly suppressed during acute VL, and restored after curative chemotherapy. The IL-6 concentration in plasma was significantly higher during active disease relative to post-chemotherapy.
- This study is the first to show the impact of VL and curative chemotherapy on drug metabolism mediated by CYP enzymes. The findings have significant implications for the prescription of drugs that are CYP3A and/or CYP2C19 substrates.

AIMS

The aim of the present study was to investigate the impact of human visceral leishmaniasis (VL) and curative chemotherapy on the activity of cytochrome P450 (CYP) 3A, CYP2C9 and CYP2C19 in patients from an endemic region in Brazil.

METHODS

Adult patients with parasitologically confirmed VL were given a CYP phenotyping cocktail, comprising midazolam, omeprazole and losartan, immediately before (Study phase 1), 2–3 days (phase 2) and 3–6 months (phase 3) after curative VL chemotherapy. CYP activity was assessed by the apparent clearance of midazolam (CYP3A), omeprazole/5-hydroxyomeprazol ratio in plasma (CYP2C19) and losartan/E3174 ratio in urine (CYP2C9).

RESULTS

Mean values (95% confidence interval) in phases 1, 2 and 3 were, respectively: log apparent midazolam clearance, 1.21 (1.10–1.31), 1.45 (1.32–1.57) and 1.35 (1.26–1.44) ml min^{-1} kg^{-1}; omeprazole/5-hydroxyomeprazole ratio, 0.78 (0.61–0.94), 0.45 (0.27–0.63) and 0.37 (0.20-0.55); losartan/E3174 ratio, 0.66 (0.39–0.92), 0.35 (0.20–0.50) and 0.35 (0.16–0.53). Analysis of variance revealed significant differences in CYP3A ($P = 0.018$) and CYP2C19 ($P = 0.008$), but not CYP2C9 ($P = 0.11$) phenotypic activity, across the three study phases.

CONCLUSION

The phenotypic activities of CYP3A4 and CYP2C19 were significantly reduced during acute VL compared with post-chemotherapy. We propose that increased plasma concentrations of proinflammatory cytokines during active disease account for the suppression of CYP activity. The failure to detect significant changes in CYP2C9 activity in the overall cohort may reflect differential effects of the inflammatory process on the expression of CYP isoforms, although the possibility of insufficient statistical power cannot be dismissed.

Introduction

Leishmania spp. are intracellular parasitic protozoa responsible for a group of neglected tropical diseases, endemic in 98 countries around the world, affecting 12 million people. Leishmaniasis has three main presentations, namely cutaneous, mucocutaneous and visceral leishmaniasis (VL or kala-azar). VL, the most severe form, has an estimated yearly incidence of 0.2 to 0.4 million cases worldwide, 90% of which occur in six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan [1, 2]. In Brazil, some 40 000 cases of VL and 2500 VL-related deaths have been reported during the first decade of the 21st century, and the disease, originally confined almost entirely to rural areas in the northeast of the country, has spread, to large cities in different regions [3, 4]. Signs and symptoms of LV include fever, cough, tiredness, weakness, loss of appetite and weight, and enlargement of the lymph nodes, liver and spleen [5]. Clinical presentation of VL results from an imbalance between host-protective and parasite escape mechanisms. Leishmania infection impairs the function of dendritic cells and macrophages. Resistance to infection is conferred by development of effective T helper 1-type responses, mounted upon release of the interleukin (IL) 12 and interferon (IFN) γ cytokine network, and boosted by proinflammatory mediators. This leads to increased circulating levels of IL-6, IL-12, IL-17, IFN-γ and tumour necrosis factor (TNF) α during active disease [6–9].

The present study explores the consequences of VL-induced systemic inflammation on cytochrome P450 (CYP)-mediated drug clearance, estimated by means of the validated phenotype probes, midazolam (CYP3A), losartan (CYP2C9) and omeprazole (CYP2C19) [10]. Expression and activity of CYP enzymes are known to be affected in the setting of high concentrations of proinflammatory cytokines in cancer and congestive heart failure [11, 12], leading to phenoconversion [13]. For human parasitic diseases there is only limited information for *Plasmodium falciparum* malaria, suggestive of reduced activity of CYP1A2 [14, 15] and CYP3A enzymes [16]. CYPs selected for the present investigation, CYP3A, CYP2C9 and CYP2C19, collectively account for the metabolism of approximately 75% of all prescribed medicines worldwide, and their expression and/or activity in human hepatocytes have been shown to be differentially regulated by inflammatory cytokines [17, 18].

Methods

This study was conducted in accordance with the revised Declaration of Helsinki and the rules of Good Clinical Practice (ICH-GCP). The clinical protocol (CAAE 0129.0.107225-10) was approved by the ethics committee of Universidade Federal de Sergipe (UFS), Aracaju, Brazil, on 8 November 2012, and all patients provided written, informed consent.

Study cohort

Twenty-four adult patients with parasitologically confirmed VL (18 men, six women), aged 18–63 years and ranging in weight from 47.0–82.7 kg, were recruited for the study. According to the 'race/colour' categorization adopted by the Brazilian Census [19], most patients ($n = 21$) self-referred as *pardo* (meaning brown), two as black and one as white. Baseline demographic and clinical characteristics are presented in Table 1. Co-medications prescribed during the study included captopril (patient 11), dipyrone (patients 16 and 18), ipratropium and fenoterol (patients 1 and 20).

VL chemotherapy

Each patient received one or two of the three anti-leishmanial drugs, namely N-methylglucamine (meglumine) antimoniate, amphotericin B deoxycholate and liposomal amphotericin B, according to the guidelines adopted by the Brazilian Ministry of Health [20], as shown in Table 1. Drug treatment was supplied free of charge to all patients through the Brazilian Public Health System.

Study design

The present open-label study comprised three phases: phase 1 (pretreatment) preceded by 2–3 days the beginning of VL chemotherapy, and phases 2 and 3 were performed 2–5 days and 3–6 months after completion of the chemotherapy, respectively. In each phase, after an overnight (>10 h) fast, the patients were admitted to a ward of the University Hospital of UFS. They were asked to empty their bladder, a catheter was introduced into a superficial vein, and a baseline (predosing) blood sample (4 ml) was collected. Patients were then given the CYP phenotyping cocktail, which consisted of oral omeprazole (20 mg capsules, Sandoz do Brasil Indústria Farmacêutica Ltda., Diadema, SP, Brazil) oral losartan (50 mg tablets, Merck Sharp & Dohme Farmacêutica Ltda., São Paulo, SP, Brazil) and oral midazolam (15 mg, Produtos Roche Químicos e Farmacêuticos, S.A., São Paulo, SP, Brazil). A standard breakfast was provided 2 h after ingestion of the phenotyping cocktail. The patients remained under observation by a physician and a protocol nurse for 8 h. Serial blood samples were drawn into heparinized tubes at zero, 15, 30 and 45 min and 1, 2, 3, 4, 5 and 6 h for CYP3A4 phenotyping and at 3 h for CYP2C19 phenotyping. The urine voided over 8 h was collected for CYP2C9 phenotyping. The blood samples were centrifuged at room temperature within 15 min after collection, and the plasma was separated and stored at −20°C. The total urine volume voided in the 8 h after administration of the phenotyping cocktail

Table 1

Demographic and clinical characteristics of the visceral leishmaniasis patients

Patients	Age (y)	Gender	Race/Colour *	Weight (kg)	Co-infections	N-methylglucamine antimoniate†	Amphotericin B deoxycholate†	Liposomal amphotericin B†
01	36	M	Brown	48.1		X	X	
02	34	M	Brown	82.7		X		
03	19	M	White	66.8			X	
04	41	M	Brown	51.4	Chagas disease	X	X	
05	28	M	Brown	62.3	Ancylostomiasis	X		
06	30	M	Brown	65.0		X		
07	41	M	Black	68.0			X	X
08	29	M	Brown	64.8			X	
09	43	M	Brown	47.0		X		
10	33	M	Brown	47.0	Ancylostomiasis	X	X	
11	53	M	Brown	68.2			X	X
12	63	M	Brown	62.0			X	X
13	25	M	Brown	85.0	Ascaridiasis	X		
14	49	M	Black	58.4			X	X
15	38	F	Brown	53		X		X
16	53	F	Brown	78.0			X	X
17	19	M	Brown	51.8		X		
18	49	M	Brown	56.4			X	
19	20	F	Brown	73.5		X		X
20	34	F	Brown	64.3				X
21	18	F	Brown	76.0				X

Abbreviations are as follows: F, female; M, male. *Self-declared, according to the 'race/colour' categories adopted by the Brazilian Census; †anti-leishmanial drugs used.

was measured, a 20 ml aliquot was separated and stored at −20°C, and the remaining volume was discarded.

Analytical procedures

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used for quantification of midazolam, omeprazole and 5-hydroxyomeprazole in plasma, and of losartan and its metabolite E-3174, in urine. The analysis of midazolam in the plasma was carried out according to the procedure described by Jabor *et al*. [21]. Briefly, aliquots of 1.0 ml plasma supplemented with clobazam (2.5 ng) as internal standard were extracted with toluene-isoamyl alcohol (100:0.1, v/v). The compounds were separated on a Purospher RP 18e column (MerckKGaA, Darmstadt, Germany) using acetonitrile: 10 mmol l^{-1} ammonium acetate aqueous solution (1:1, v/v) as the mobile phase. The protonated ions and their respective ion products were monitored $m > z$ at $301 > 259$ for the internal standard and at $326 > 291$ for midazolam. The quantification limit for midazolam was 0.1 ng ml^{-1} (coefficient of variation [CV] = 13.4%) and the method was linear in the 0.1–1000 ng ml^{-1} range.

The analysis of omeprazole and 5-hydroxyomeprazole in the plasma was performed as previously reported by Rocha *et al*. [22]. Aliquots of 0.5 ml plasma containing the internal standard antipyrine (6.25 μg) were extracted with dichloromethane:ethyl ether (95:5, v/v). The drugs were separated on an RP-select B column using a mixture of acetonitrile and 0.1% formic acid (27:73, v/v). The species were monitored at the transitions $346 > 198$ for omeprazole, $362 > 214$ for 5-hydroxyomeprazole and at $189 > 104$ for antipyrine. The quantification limit for both omeprazole and 5-hydroxyomeprazole was 1.0 ng ml^{-1}, the CVs were 11.7% and 12.8%, respectively, and the method was linear for both analytes in the 1–1000 ng ml^{-1} range.

The method of analysis of losartan and its metabolite losartan carboxylic acid (E3174) in the urine was developed and validated using LC-MS/MS. Two aliquots of 100 μl urine containing the internal standard antipyrine (0.5 μg), 200 μL of 1 mol l^{-1} citrate buffer (pH = 5.0) and 2 ml methyl *tert*-butyl ether were added and then the samples were vortexed for 30 s. The organic layers were separated, transferred to other tubes and evaporated to dryness. The residues were reconstituted in the mobile phase, consisting of a mixture of acetonitrile:0.2% acetic acid (50:50, v/v), and the separation was performed on a LiChrospher® 100 RP-8 column (MerckKGaA, Darmstadt, Germany). Electrospray ionization was performed in positive ion mode, monitoring the transitions $423 > 207$ for losartan, $437 > 234$ for

the metabolite E3174 and 189 > 104 for the internal standard antipyrine. The quantification limit for losartan was 5 ng ml^{-1} (CV 8.5%) and the method was linear in the 5–2000 ng ml^{-1} range. For the metabolite E3174, the quantification limit was 2.5 ng ml^{-1} (CV 5.9%) and the method was linear in the 2.5–1000 ng ml^{-1} range.

IL-6 concentrations in the human plasma were determined by enzyme-linked immunosorbent assay (ELISA) (Duo Set System, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's recommendations. The detection limit of the assay was 1 pg ml^{-1}.

Estimation of CYP activity

CYP3A activity was estimated by the apparent clearance (CL/*F*) of midazolam, corrected for body weight and expressed as ml min^{-1} kg^{-1}: CL/*F* = dose/AUC$_{0-\infty}$, where AUC$_{0-\infty}$ is the area under the plasma concentration–time curve from time 0 to infinity. The software WinNonlin 4.4 (Pharsight Corporation, Mountain View, CA, USA) was used in these calculations. Midazolam CL/*F* has been shown to vary widely (29–48-fold) among healthy individuals, although most individuals (84%) have CL/*F* values within the 10–40 ml min^{-1} kg^{-1} range [23]. The ratio of omeprazole/5-hydroxyomeprazole concentrations in plasma at 3 h was used to assess the activity of CYP2C19. A log ratio >0.6 was set as the cutoff value for poor CYP2C19 metabolizers [24]. CYP2C9 activity was estimated by the losartan/E3174 concentration ratio in urine voided over 8 hours. A log ratio > 1.96 was set as the hypothetical cutoff value for poor CYP2C9 metabolizers [25].

Statistical analyses

Statistical analyses were performed after logarithmic transformation of the midazolam CL/*F* corrected for body weight (ml min^{-1} kg^{-1}), the omeprazole/5-hydroxyomeprazole and losartan/E3174 concentration ratios and the IL-6 concentration in the plasma. Differences across the three study phases were assessed by the repeated-measures analyses of variance (ANOVA) test. When a significant difference was detected by ANOVA, pair-wise comparisons between phases were carried out using the *t*-test with Bonferroni's correction. Values of $P < 0.05$ were considered to indicate statistically significant differences.

Sample size determination

Based on previous data for Brazilians, we calculated that 21 patients were required to have 80% power ($\beta = 0.2$) to detect, at the 5% significance level ($\alpha = 0.05$), a 25% difference in CYP3A activity measured before VL chemotherapy *vs.* the activity measured in each phase after chemotherapy.

Results

The 24 patients enrolled in the study had clinical evidence of active infection, including fever, anaemia, weight loss and hepatosplenomegaly, but no haemorrhagic manifestations. Three patients did not complete the study protocol and were excluded from the statistical analyses. Baseline demographical characteristics of the 21 patients included in these analyses, and their VL chemotherapy are shown in Table 1. Drug treatment led to marked clinical improvement, including reversal of hepatosplenomegaly, and a significant increase in body weight and in haematological parameters, especially white blood cell and platelet counts (Table 2). There were no adverse events related to the CYP probe drugs that required medical intervention. As anticipated, midazolam-induced sedation and sleepiness occurred in most patients.

Summary data for the pharmacokinetic parameters of the three CYP phenotypic probes, in each study phase, are presented in Table 3. Figures 1-3 show box-plots of the log-transformed phenotypic data and results of the

Table 2

Haematological parameters of visceral leishmaniasis patients before and after drug treatment

Parameter*	Phase 1 Pretreatment	Phase 2 Post-treatment	Paired *t*-test *P* value
Body weight (kg)	63.3 ± 10.9	66.4 ± 11.2	<0.0001
Hgb (g dl^{-1})	8.4 ± 1.8	10.2 ± 2.0	0.001
Hct (%)	26.3 ± 5.0	31.5 ± 5.9	0.001
Leukocytes (mm^3)	2045 ± 995	4376 ± 1891	<0.0001
Platelets (mm^3)	119 217 ± 57,460	213 400 ± 40 771	<0.0001

Abbreviations are as follow: Hgb, haemoglobin; Hct, haematocrit. *Data are expressed as mean ± standard deviation.

Table 3

Phenotype assessment of cytochrome P450 isoenzymes (CYPs) in visceral leishmaniasis patients

CYP phenotypic index	Study phase	Median (interquartile range)
CYP3A apparent midazolam clearance (ml min^{-1} kg^{-1})	1	19.5 (13.5–25.4)
	2	36.5 (22.7–50.3)
	3	26.5 (19.2–33.8)
CYP2C19 omeprazole/5-hydroxyomeprazole ratio in plasma	1	8.3 (5.4–11.2)
	2	4.7 (2.2–7.1)
	3	3.8 (2.0–5.6)
CYP2C9 losartan/E3174 ratio in urine	1	19.4 (1.4–37.4)
	2	3.5 (1.5–5.6)
	3	4.6 (1.0–8.1)

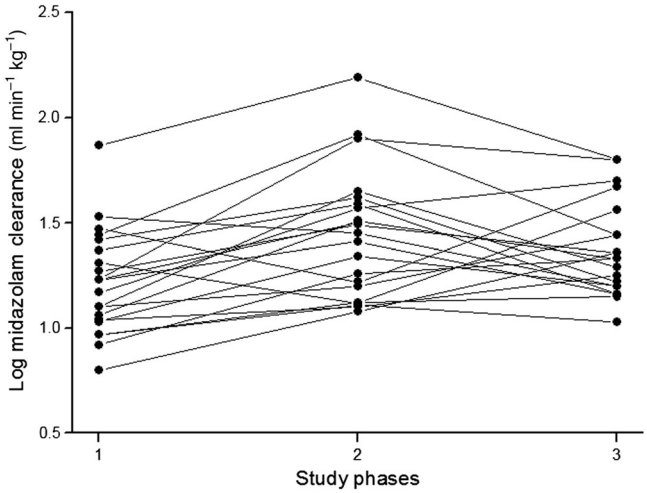

Figure 1

Individual values of the apparent midazolam clearance in visceral leishmaniasis (VL) patients, before (phase 1), 2–3 days (phase 2) and 3–6 months (phase 3) after curative VL chemotherapy. Midazolam (15 mg) was administered orally, as part of a cytochrome P450 phenotype cocktail

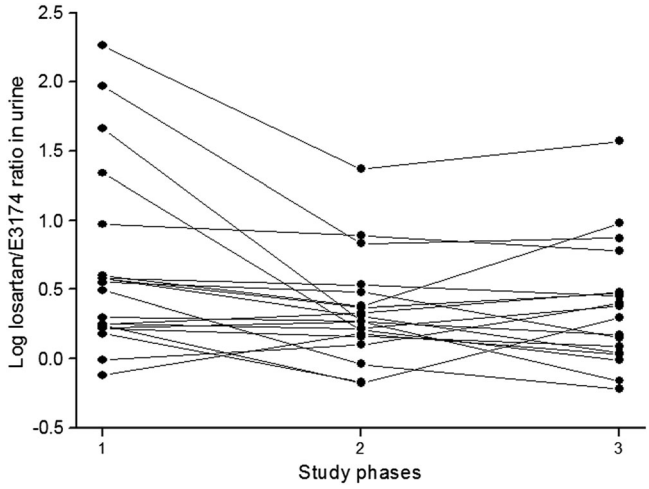

Figure 3

Individual values of the losartan/E-3174 ratio in the urine of visceral leishmaniasis (VL) patients, before (phase 1), 2–3 days (phase 2) and 3–6 months (phase 3) after curative VL chemotherapy. Losartan and E-3174 concentrations were measured in the urine voided over 8 h after oral administration of losartan (50 mg)

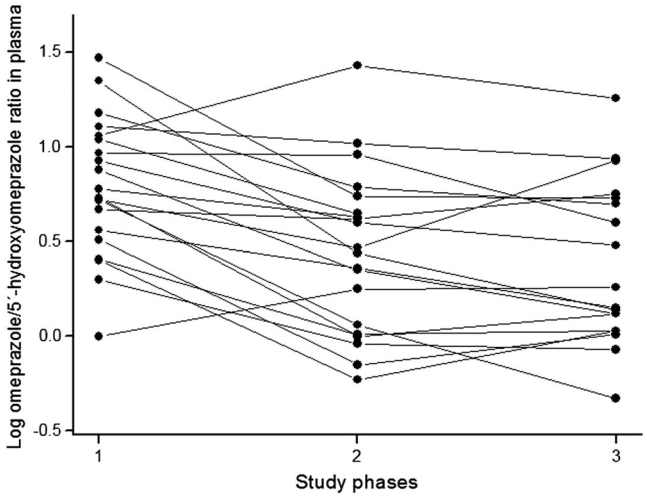

Figure 2

Individual values of the omeprazole/5-hydroxyomeprazole ratio in the plasma of visceral leishmaniasis (VL) patients, before (phase 1), 2–3 days (phase 2) and 3–6 months (phase 3) after curative VL chemotherapy. Omeprazole and 5-hydroxyomeprazole concentrations were measured in blood samples collected 3 h after oral administration of omeprazole (20 mg)

ANOVA statistical analyses. We will next describe the findings for each CYP, according to the data presented in Table 3 and Figures 1–3.

CYP3A activity

During the active disease (phase 1), the oral CL/*F* of midazolam varied 17-fold (4.5–78.4 ml min^{-1} kg^{-1}). Clearance was within the 10–40 ml min^{-1} kg^{-1} range in 16 patients (76%), below 10 ml min^{-1} kg^{-1} in four patients and exceeded 40 ml min^{-1} kg^{-1} in one patient. After completion of VL chemotherapy (phase 2), the midazolam CL/*F* remained above 40 ml min^{-1}/kg^{-1} in one patient, and was within 10–40 ml ml^{-1} kg^{-1} in all other patients. The same distribution was observed when the patients were phenotyped 3–6 months later (phase 3). The ANOVA test revealed significant differences in the midazolam CL/*F* across the three study phases ($P = 0.018$). Post-hoc Bonferroni tests showed significantly greater midazolam CL/*F* in phase 2, compared with phase 1 ($P = 0.03$), a trend towards increased CL/*F* in phase 3 relative to phase 1 ($P = 0.09$) and no difference between phases 2 and 3 ($P > 0.5$; Figure 1 and Table 3).

CYP2C19 activity

Before LV chemotherapy, the ratio of omeprazole/5-hydroxyomeprazole concentrations in plasma at 3 h ranged from 0.5 to 29.4. Using a cutoff value of >0.6 for the log omeprazole/5-hydroxyomeprazole ratio [24], 14 patients were classified as poor metabolizers. Five of these patients converted to the extensive metabolizer phenotype after VL chemotherapy (phase 2) and remained extensive metabolizers in the subsequent 3–6 months (phase 3). The log omeprazole/5-hydroxyomeprazole ratio differed significantly across the three study phases (ANOVA $P = 0.008$). Post-hoc Bonferroni tests showed significantly greater ratios in phases 2 and 3, compared with phase 1 ($P = .04$ and $P = 0.01$, respectively) but no difference between phases 2 and 3 ($P > 0.5$; Figure 2 and Table 3).

Table 4

Concentration of interleukin 6 (IL-6) in the plasma of visceral leishmaniasis patients

Study phase	Il-6 concentration (pg ml^{-1})*
1	26.5 (15.9–84.9)
2	4.5 (1.0–14.5)
3	1.0 (1.0–16.1)

*Data presented as median (interquartile range).

CYP2C9 activity

The ratio of losartan to E3174 in the urine varied over wide ranges both before (0.75–181.5) and in the two phases after (0.25–37.1) LV chemotherapy (Table 3). Using a cutoff value of 1.96 for the log losartan/E3174 ratio [25], two patients were classified as poor metabolizers in phase 1. Both patients converted to the extensive metabolizer phenotype in phase 2, and all patients remained extensive metabolizers in phase 3. The repeated-measures ANOVA test failed to detect significant difference in urinary losartan/E3174 log ratio across the three study phases in the overall patient cohort ($P = 0.11$). Of note, however, VL chemotherapy caused a marked decrease in the log losartan/E3174 ratio in the four patients who had the largest ratios (>1.0, Figure 3) during acute disease.

IL-6 measurements

All except one patient had IL-6 concentrations in the plasma above the lower detection level (1 pg ml^{-1}) in phase 1, compared with six and 11 patients in phases 2 and 3, respectively. Summary data of the IL-6 concentrations in the three study phases are shown in Table 4. The ANOVA test revealed a significant difference in log-transformed IL-6 concentrations across the three study phases ($P < 0.0001$). Post-hoc Bonferroni tests showed significantly lower IL-6 concentrations in phases 2 and 3 compared with phase 1 ($P < 0.0001$ for both comparisons), but no significant difference between phases 2 and 3 ($P = 0.33$).

Discussion

This was the first investigation of the activity of CYP enzymes during active human VL and following curative anti-leishmaniasis chemotherapy. The study cohort comprised adult patients with confirmed LV, recruited in an endemic area in the northeast of Brazil, who were treated according to the guidelines recommended by the Brazilian Ministry of Health and were phenotyped for CYP activity using established protocols. Phenotypic probes selectively or preferentially metabolized by CYP3A (midazolam), CYP2C19 (omeprazole) and CYP2C9 (losartan) were administered during active disease and after VL chemotherapy, and their metabolic ratios (omeprazole and losartan) or oral CL/*F* corrected for body weight (midazolam) were used as indices of the respective CYP activity [10]. These indices pointed to significantly lower activity of CYP3A and CYP2C19 during active disease compared with the activity measured after curative chemotherapy.

Parallel measurements of the IL-6 concentration in plasma showed significantly higher values during active disease relative to postchemotherapy. The range of IL-6 plasma concentrations during acute disease was in good agreement with recently reported data for Brazilian VL patients without haemorrhagic manifestations [8]. Costa *et al*. [8] concluded that IL-6 plays a major role in the pathogenesis of VL, not only because it is directly and independently associated with several pathological traits of the disease, but also by its ability to induce other disease-causing cytokines, such as IL-1β, IFN-γ and IL-8. Accordingly, strong correlation was observed between the plasma concentrations of these cytokines and that of IL-6 in Brazilian LV patients [8]. This finding was verified in Sudanese patients harbouring both leishmanial (*Leishmania donovani*) and malarial (*P. falciparum*) parasites [9].

We propose that the increased plasma concentrations of IL-6 and other proinflammatory cytokines may account for the reduced activity of CYP3A and CYP2C19 during active VL disease. Several observations support this interpretation. First, human hepatocyte *in vitro* models have consistently shown a reduction in CYP activity and expression by proinflammatory cytokines, notably IL-6, IL-1, INF-γ and TNF-α [18,26–28]. Second, distinct CYPs show differential responses to cytokines; for example, a recently published review lists CYP3A4 and CYP2C19, but not CYP2C9, as being downregulated by IL-6, whereas IL-1 is linked to the downregulation of CYP3A4, with no effect on CYP2C9 or CYP2C19, and TNF-α to the decreased activity of CYP2C19 [18]. Dickmann *et al*. [29] compared the potency and extent of IL-6-induced downregulation of CYP3A4, CYP2C9 and CYP2C19 in a human hepatocyte culture model, and reported that CYP3A4 was the most sensitive [half maximal effective concentration (EC_{50}) = 3.2 pg ml^{-1}] and CYP2C9 the least sensitive (EC_{50} = 112 pg ml^{-1}). Furthermore, IL-6 induced the complete suppression of CYP3A4 but submaximal suppression of CYP2C9 and CYP2C19. Third, a monoclonal antibody directed against IL-6 was shown to neutralize IL-6 activity and cause CYP desuppression in human hepatocytes [29]. Fourth, *in vivo* CYP3A4 downregulation by IL-6 was reversed by tocilizumab, an IL-6 receptor inhibitor used for the treatment of rheumatoid arthritis (RA). Thus, exposure of RA patients to simvastatin (a CYP3A4 substrate) was significantly reduced after treatment with tocilizumab,

which was explained by 'normalization of CYP3A4 activity to a level similar to that in healthy persons, thereby decreasing exposure to drugs metabolized by CYP3A4' [30]. Collectively, these findings may explain: (i) the reduced phenotypic activity of CYP3A and CYP2C19 in VL patients, as a consequence of systemic inflammation and high plasma concentrations of IL-6 and other cytokines; and (ii) the increase in CYP3A and CYP2C19 activity following curative chemotherapy and the decline in the plasma concentration of IL-6 and, most likely, other cytokines.

In contrast to CYP3A and CYP2C19, CYP2C9 phenotypic activity did not differ significantly across the study phases in the overall patient cohort. This may be explained by the differential effect of cytokines on individual CYPs (see above). According to Morgan and coworkers: '…of the inflammatory cytokines and CYP isoenzymes investigated, the concentration–inhibition relationship between IL-6 and CYP3A4 appears to be the strongest' and it might be predicted that most infections or diseases associated with inflammation will downregulate CYP3A4, whereas the extent of suppression of CYP2C9 and CYP2C19 may depend on the nature of the inflammatory condition and the combined effect of increased plasma concentrations of multiple cytokines [17, 18]. However, we cannot dismiss the possibility of insufficient statistical power to detect changes in CYP2C9 phenotypic activity because of the wide variation in the urinary losartan/E3174 log ratio across the study phases. In this context, it is noteworthy that VL chemotherapy caused a marked decrease in the log losartan/E3174 ratio in the four patients who presented the largest ratios during acute disease (Figure 3). Indeed, two of these patients were classified as poor CYP2C9 metabolizers in phase 1, and both converted to the extensive metabolizer phenotype after VL chemotherapy.

To our knowledge, changes in CYP activity in other human parasitic diseases have been investigated only in malaria. Akinyinka *et al*. [14, 15] studied the pharmacokinetics of caffeine and its metabolites in Nigerian children and adults suffering from uncomplicated *P. falciparum* malaria, and concluded that CYP1A2 activity was lower in these patients. Pukrittayakamee *et al*. [16] reported a reduction in the clearance of quinine in adult Thai patients with severe or moderately severe *P. falciparum* malaria. This effect was thought to result predominantly from a disease-induced dysfunction of hepatic CYP3A activity, which impaired the conversion of quinine into its major metabolite, 3-hydroxyquinine. In murine malaria models, downregulation of the CYP isoenzymes has been verified in liver microsomes of *Plasmodium berghei*-infected mice, and was associated with prolongation of midazolam sleeping time and a slower clearance of chlorzoxazone [31–33].

In conclusion, this was the first investigation of the impact of VL and curative chemotherapy on the activity of three major CYP isoenzymes, which collectively account for the metabolism of approximately 75% of all prescribed medicines worldwide. We report that the phenotypic activities of CYP3A (assessed by the CL/*F* of midazolam) and CYP2C19 (assessed by the metabolic ratio of omeprazole/5-hydroxyomeprazol in plasma) were suppressed in the acute phase of VL and restored after curative chemotherapy. We propose that the increased plasma concentrations of IL-6 and other proinflammatory cytokines may account for the suppressed activity of CYP3A4 and CYP2C19 during active VL disease; decline of the cytokine levels following curative chemotherapy restores the activity of these two CYP isoforms. The failure to detect significant changes in CYP2C9 activity (assessed by the ratio of losartan/E3174 in urine) is thought to reflect the differential effects of inflammatory cytokines on the expression of CYP isoforms, although the possibility of insufficient statistical power should not be dismissed.

Competing Interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf, and declare: GS-K had grant support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ); GS-K, AB and MB-N had grant support from Fundação de Amparo à Pesquisa do Estado da Bahia ((PNX0019/2009 CNPq-FAPESB – PRONEX); the other authors had no support from any organization; all authors had no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

We thank Dr Patrícia Bozza and Mr Edson Assis for carrying out the quantification of IL-6 in the plasma samples.

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