

Hepatotoxicity of oral and intravenous voriconazole in relation to cytochrome P450 polymorphisms

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Objectives: Voriconazole, like all other antifungals of the azole group, is potentially hepatotoxic. A large interpatient variability of liver enzyme elevations during oral or intravenous (iv) voriconazole administration is observed. This interpatient variability may be explained by differences in voriconazole metabolism because of cytochrome P450 polymorphisms. We examined the relationship between cytochrome P450 polymorphisms and hepatotoxicity in immunocompromised patients predominantly receiving oral formulations of voriconazole.

Methods: In a single institution retrospective study of 86 immunocompromised patients receiving oral ($n = 74$) or iv ($n = 12$) voriconazole, we studied the influence of cytochrome P450 polymorphisms (CYP2C19, CYP2C9 and CYP3A5) on the maximum bilirubin and serum liver enzyme levels [alkaline phosphatase, gamma-glutamyl transpeptidase (GGT), serum aspartate aminotransferase and serum alanine aminotransferase] and their respective common toxicity criteria scores (CTC-scores).

Results: Median serum bilirubin as well as the level of all other liver enzymes increased during voriconazole treatment. A decline in CTC-score was observed in zero (0%) to six (7%) patients; an increase in CTC-score was demonstrated in 36 (42%) to 54 (63%) patients. No statistically significant differences in maximum value or maximum increase of liver enzymes or CTC-score in relation to cytochrome P450 polymorphisms were observed. Only a trend towards higher maximum CTC-score of GGT for wild-type of CYP2C9 was observed ($P = 0.046$).

Conclusions: No significant relationship between CYP2C9, CYP2C19 or CYP3A5 polymorphisms and serum liver enzyme levels was observed in patients treated with voriconazole.

Keywords: liver, leukaemia, transplantation, pharmacokinetics

Introduction

Voriconazole is a triazole antifungal agent with activity against most yeasts and moulds that cause disease in humans.^{1,2} Among the most frequently observed side effects of voriconazole are visual disturbances and liver enzyme elevation.³ We recently demonstrated that in daily practice, liver enzyme elevations may be more frequent than previously observed in clinical trials. Liver enzyme elevations were observed in 36.4% to 68.6% in

this study, in which voriconazole was given orally from the beginning in the majority of patients.⁴ Previous studies demonstrated lower rates of hepatotoxicity ranging from 11.7% to 18.9%.^{5,6}

N-oxidation of voriconazole into its major circulating metabolite is predominantly performed by the cytochromes CYP2C9, CYP2C19 and CYP3A4.⁷ Ikeda *et al.*⁸ subsequently postulated that genotype status and/or co-administration of drugs that modulate CYP2C9, CYP2C19 or CYP3A4 activity could affect

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voriconazole plasma levels, which was partly confirmed for CYP2C19. In addition, voriconazole plasma levels of >6000 ng/mL are correlated with liver function abnormalities or liver failure.^{6,9} This has led to the postulation that cytochrome P450 polymorphisms, correlated with decreased enzyme activities, can predispose to more serious hepatotoxicity of voriconazole administration.

We retrospectively analysed the frequency of elevated liver enzymes in a population predominantly receiving oral voriconazole and correlated this with the presence or absence of genetic polymorphisms of CYP2C9, CYP2C19 and CYP3A5. CYP3A5 instead of CYP3A4 was studied because of the low frequency of CYP3A4 in the Dutch population and the large overlap in enzyme activity of CYP3A5 and CYP3A4.

Materials and methods

Patients

All patients with a haematological malignancy receiving voriconazole from 1 November 2002 to 1 January 2005 at the Erasmus MC, Rotterdam, The Netherlands were studied. Twelve patients were treated first with the standard 7 day intravenous (iv) and sequential oral administration of voriconazole.² All other patients received oral voriconazole from the start: 6 mg/kg twice daily for the first 24 h, 4 mg/kg twice daily from days 2 to 7 and 200 mg twice daily thereafter.

Ethics

Patients in this study were included in prospective randomized trials, in which samples are withdrawn systematically. Because of this, and the retrospective character of the study, we rely on a 'no-objection' statement of the patients for the blinded cytochrome polymorphism measurement. Patient characteristics and liver enzyme results have been gathered earlier in a coded database. The results of the cytochrome polymorphisms were coded in another database. In this way, relations between the patient's anonymized information and the coded cytochrome polymorphisms were investigated.

Toxicity registration

Bilirubin, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) concentrations were measured three times a week during voriconazole therapy and recorded as absolute values and according to the NCI Common Terminology Criteria for adverse events (CTCAE version 2.0, <http://ctep.cancer.gov>). If hepatotoxicity persisted after discontinuation of voriconazole, measurement was continued until concentrations decreased. We classified hepatotoxicity according to absolute liver enzyme values and to common toxicity criteria (CTC) grades [for bilirubin grade 0, none; grade 1, >ULN (upper limit of normal)–1.5 × ULN; grade 2, >1.5–3.0 × ULN; grade 3, >3.0–10.0 × ULN and grade 4, >10.0 × ULN and for ALP, GGT, AST and ALT grade 0, none; grade 1, >ULN–2.5 × ULN; grade 2, >2.5–5.0 × ULN; grade 3, 5.0–20.0 × ULN and grade 4, >20.0 × ULN].

Cytochrome P450 polymorphisms

For every patient, the CYP2C19, CYP2C9 and CYP3A5 genetic polymorphisms were determined on 5 ng of genomic DNA, using LightCycler assays (CYP2C9 and CYP2C19) or by polymerase

chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay (CYP3A5).^{10,11}

The LightCycler assay was performed by using fluorogenic hybridization probes (TIB MOLBIOL, Berlin, Germany) and a LightCycler–FastStartDNA Master Hybridization kit (Roche, Mannheim, Germany). Hybridization of the probes to the target DNA resulted in fluorescence resonance energy transfer between two fluorophores. During melting of the final product, the sequence alteration is detected as a change in the melting temperature of the sensor probe.¹⁰

The PCR-RFLP was performed by adding a buffer, deoxynucleotide triphosphates (Roche), AmpliTaq Gold (Perkin-Elmer, MA, USA) and the forward and reverse primers to the PCR mixture. The underlined nucleotides are mismatches with the CYP3A5 sequence, creating restriction sites in the PCR product. The PCR product was then digested with the appropriate restriction enzyme (Roche) and subsequently analysed on a 4% agarose/Tris–borate–EDTA gel with ethidium bromide staining.¹¹

CYP2C19 and CYP2C9 were scored as 'wild-type' if no *2 or *3 alleles were detected. CYP3A5 was scored as 'expressers' if no or only one *3 allele was detected.

Statistical analyses

The maximum liver enzyme value during voriconazole therapy, the absolute increase from baseline, the maximum CTC-score and the increase from baseline CTC-score to maximum CTC-score were compared in patients with and without wild-type cytochrome polymorphism using the Kruskal–Wallis test. The incidence of a CTC-score increase of ≥2 in patients with and without wild-type cytochrome polymorphism was calculated and compared with the χ^2 test or Fisher's exact test whichever was appropriate. All *P* values are two-sided. Owing to the large number of tests performed, only *P* values less than 0.01 were considered statistically significant, whereas *P* values less than 0.05 were denoted as a trend.

Results

Liver enzyme abnormalities

A total of 86 consecutive patients were included (Table 1). Twelve patients were treated intravenously during the first week

Table 1. Characteristics of 86 patients included in the study

Characteristics	Category	Number
Total		86
Median age (range)	years	56 (17–84)
Sex	male	54 (63%)
	female	32 (37%)
Reason of voriconazole	possible IA	39 (45%)
	probable IA	33 (38%)
	proven IA	1 (1%)
	invasive <i>Candida</i>	8 (9%)
	other	5 (6%)
Reason for hospitalization	acute leukaemia treatment	51 (59%)
	transplantation	15 (17%)
	other	20 (23%)

IA, invasive aspergillosis.

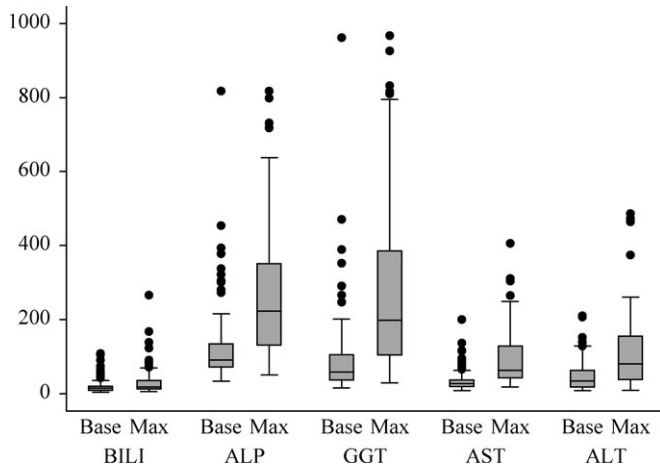


Figure 1. Box-and-whisker plot of baseline (Base) and maximum (Max) liver enzymes values during treatment with voriconazole. y-axis values are in mmol/L for bilirubin (BILI) and in U/L for ALP, GGT, AST and ALT. Two extreme maximum values (GGT of 2121 and AST of 2192) have been removed from this graph for reasons of clarity of the figure.

and 74 patients were treated orally. Median baseline concentrations for the 86 patients were 14 mmol/L (range 3–108) for bilirubin (ULN 16 mmol/L) and 91 (33–818) U/L (ULN 119 U/L), 58 (15–962) U/L (ULN 35), 27 (8–200) U/L (ULN 36) and 35 (7–210) U/L (ULN 40), respectively, for ALP, GGT, AST and ALT (Figure 1). Median baseline CTC values were 1 for GGT and 0 for all others.

During voriconazole therapy, the median maximum concentrations were 18 (range 5–266) mmol/L and 223 (50–818), 200 (29–2121), 62 (18–2192) and 81 (9–486) U/L, respectively, for bilirubin, ALP, GGT, AST and ALT (Figure 1). A decline in CTC-score was observed in six (7%), two (2%), two (2%), zero (0%) and five (6%) patients for bilirubin, ALP, GGT, AST and ALT. An increase in CTC-score was found in 31 (36%), 53 (62%), 52 (60%), 54 (63%) and 45 (52%) patients for bilirubin, ALP, GGT, AST and ALT. The median maximum increase

regarding CTC values was 0, 1, 1, 1 and 1, respectively, for bilirubin, ALP, GGT, AST and ALT. A maximum CTC-score of 3 or more was observed in 16 (19%), 9 (10%), 41 (48%), 15 (17%) and 19 (22%), respectively, for bilirubin, ALP, GGT, AST and ALT. An increase in CTC-score of 2 or more was observed in 16 (19%), 17 (20%), 37 (43%), 27 (31%) and 26 (30%) of the 86 patients, respectively, for bilirubin, ALP, GGT, AST and ALT.

Cytochrome P450 polymorphisms

The wild-type CYP2C19 and CYP2C9 genotypes were found in 63 (73%) and 57 (66%) of patients, whereas CYP3A5 expressers were found in 23 (27%) of 86 patients (Table 2). CYP3A5 polymorphism determination failed twice, which left 84 patients for the analysis. No significant relationship was found between wild-type of CYP2C19 and CYP2C9 or CYP3A5 expressers and the maximum value in the concentration of bilirubin, ALP, GGT, AST and ALT ($P = 0.12–0.97$). No significant relationship was found between wild-type of CYP2C19 and CYP2C9 or CYP3A5 expressers and the increase in the concentration of bilirubin, ALP, GGT, AST and ALT ($P = 0.23–0.90$). In addition, no significant relationship was found between wild-type of CYP2C19 and CYP2C9 or CYP3A5 expressers and the maximum CTC-score of bilirubin, ALP, ALT and AST ($P = 0.17–0.94$). Only the maximum CTC-score of GGT tended to be higher for CYP2C9 wild-type ($P = 0.046$), but not for wild-type of CYP2C19 and CYP3A5 expressers. No significant relationship between an increase of 2 or more points in the CTC-score for bilirubin, ALP, GGT, AST and ALT could be demonstrated for wild-type CYP2C9, CYP2C19 or CYP3A5 expressers ($P = 0.14–1.00$). In addition, no significant relationship was established for wild-type CYP2C9, CYP2C19 or CYP3A5 expressers and a maximum CTC grade of 2 or more for bilirubin, ALP, ALT and AST ($P = 0.15–0.99$). The maximum CTC-score of 2 or more tended to be higher for CYP2C9 wild-type ($P = 0.049$), but not for wild-type of CYP2C19 and CYP3A5 expressers. Subgroup analysis for the oral and iv administration of voriconazole revealed no significant

Table 2. Number of patients with a 2 or more increase of CTC-score of liver enzyme after the start of voriconazole treatment ($=\Delta\text{CTC} > 1$) according to cytochrome P450 polymorphisms

Cytochrome		$\Delta\text{CTC} > 1$				
		Bili	ALP	GGT	AST	ALT
CYP2C19 ($n = 86$)	WT ($n = 63$)	14 (22%)	13 (21%)	25 (40%)	19 (30%)	18 (29%)
	non-WT ($n = 23$)	2 (9%)	4 (17%)	12 (52%)	8 (35%)	8 (35%)
	<i>P</i> value	0.22	1.00	0.34	0.79	0.60
CYP2C9 ($n = 86$)	WT ($n = 65$)	10 (15%)	13 (20%)	27 (42%)	16 (25%)	14 (22%)
	non-WT ($n = 21$)	6 (29%)	4 (19%)	10 (48%)	11 (52%)	12 (57%)
	<i>P</i> value	0.78	0.40	0.26	0.46	0.14
CYP3A5 ($n = 84$)	Expr. ($n = 23$)	3 (13%)	2 (9%)	9 (39%)	8 (35%)	7 (30%)
	non-expr. ($n = 61$)	13 (21%)	15 (25%)	28 (46%)	19 (31%)	19 (31%)
	<i>P</i> value	0.54	0.14	0.63	0.59	1.00

WT, wild-type; expr., expresser; Bili, bilirubin.

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differences with regard to the liver enzymes and the investigated cytochrome P450 polymorphisms (this subgroup analysis was powered only to detect large differences between iv and oral administration).

Discussion

The postulated influence of CYP3A5 genotype status on the voriconazole-induced liver enzyme elevations could not be confirmed in this study.^{7,9} The only trend that was found was a higher maximum CTC-score for GGT and wild-type CYP2C9. The absence of a relationship may be caused by the relatively limited number of patients in this study and therefore lack of power to detect moderate but possibly clinically relevant differences. Alternatively, CYP3A5 polymorphisms may be of less impact in the interpatient variability of liver enzyme toxicity because of the fact that the metabolism of voriconazole *in vivo* may differ from the results of *in vitro* observations. In the case of redundancy, other enzymes may compensate for the absence of CYP3A5 activity in the metabolism of voriconazole. Especially, the oral administration of voriconazole may have another hepatotoxicity profile than the iv administration, although the systemic voriconazole plasma levels may be similar.¹² Similar explanations for the lack of relation of liver enzymes and cytochrome P450 polymorphisms for CYP2C19 and CYP2C9 can be stated. This may be in concordance with recent results of observations in patients with non-wild-type CYP2C9 status.^{13,14} In these two reports, contradictory results of the effect of CYP2C9 and voriconazole plasma concentrations are reported.

This study again demonstrates the higher incidence of hepatotoxicity of voriconazole in clinical practice than reported in the literature. In particular, CTC-scores of 3 or more, which may require temporary withdrawal of voriconazole, were demonstrated in 10% to 48% of patients depending on the measured liver enzyme. The higher incidence of hepatotoxicity of voriconazole may be explained by the oral administration of the loading dose during the first week in contrast to most previous studies. In a smaller previous study, however, we could not demonstrate this postulated difference between oral and iv therapy.⁴ Gastrointestinal absorption of the oral 7 day loading dose regimen will result in high systemic concentrations and especially high serum concentrations locally in the portal vein, predominantly during the first day.¹⁵ This may lead to higher voriconazole concentrations in the liver during the first week of therapy and subsequent higher incidence of liver enzyme abnormalities.

This study was hampered by the lack of voriconazole serum concentrations. This lack was caused by the retrospective character of the study. In a prospective study, serum samples can be measured and correlated with cytochrome P450 polymorphisms and liver enzyme levels. In this way, a more physiological insight into the voriconazole-induced hepatotoxicity can be obtained.

In conclusion, in patients on predominantly oral voriconazole therapy, hepatotoxicity is observed frequently. Cytochrome P450

polymorphisms (CYP3A5, CYP2C19 and CYP2C9) did not predict voriconazole-induced hepatotoxicity.

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Transparency declarations

None to declare.

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