Voriconazole potentiates warfarin-induced prothrombin time prolongation

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Aims Voriconazole is a novel triazole with broad-spectrum antifungal activity. It is likely that some patients receiving voriconazole may also require treatment with the anticoagulant warfarin. Cytochrome P450 isoenzymes are important in the metabolism of both these drugs. This study investigated the effect of voriconazole on the pharmacodynamics of warfarin by measuring prothrombin time, and also evaluated the safety and tolerability of the coadministered drugs.

Methods This was a double-blind, placebo-controlled, two-way crossover study in which healthy male subjects received either 300 mg voriconazole or placebo twice daily on days 1–12, plus a single oral dose of 30 mg warfarin on day 7 of each study period. Volunteers were randomized to one of the following treatment sequences: voriconazole + warfarin followed by placebo + warfarin or placebo + warfarin followed by voriconazole + warfarin. There was a washout of at least of 7 days between treatment periods.

Results The mean $C_{\max}$, AUC_{τ} and $t_{\max}$ for voriconazole were 3736 ng ml⁻¹, 25 733 ng·h ml⁻¹, and 1.66 h, respectively. Both the mean maximum change from baseline prothrombin time and the mean area under the effect curve (AUEC) for prothrombin time during coadministration with voriconazole (17 s and 3211 s·h, respectively) were statistically significantly greater than the mean values observed during the placebo period (8 s and 2282 s·h). Prothrombin times were still increased by a mean value of 5.4 s 144 h post warfarin dose following coadministration with voriconazole compared with a mean value of 0.6 s in the placebo treatment period.

Conclusions Coadministration of voriconazole and warfarin potentiates warfarin-induced prothrombin time prolongation. Regular monitoring of prothrombin time is recommended if these drugs are coadministered, with appropriate adjustment of the dose of warfarin.

Keywords: drug interaction, pharmacodynamics, prothrombin, voriconazole, warfarin

Introduction

Voriconazole is a new triazole antifungal agent, available as oral and intravenous formulations, with potent activity against a broad spectrum of clinically significant pathogens, including *Aspergillus* and *Candida* species [1–3], and emerging fungal pathogens, such as *Scedosporium* and *Fusarium* species [4, 5].

The pharmacokinetics of voriconazole have been investigated following single and multiple (10–30 days)

doses in both healthy volunteers and patients [6–8]. Voriconazole is extensively metabolized by the cytochrome (CYP) P450 system, mainly by the polymorphically expressed CYP2C19 isoenzyme, by CYP2C9, and to a lesser extent by CYP3A4 [9].

The S-form of the oral coumarin anticoagulant warfarin is metabolized predominantly by CYP2C9, and has been reported to interact with numerous classes of drug, including some antifungal agents [10]. These reports predominantly involve miconazole [11] and fluconazole [12], although there have also been reports of interactions with terbinafine [13, 14]. However, in a subsequent controlled study, terbinafine produced no change in either the pharmacokinetics or pharmacodynamics of warfarin, indicating a lack of effect of terbinafine on warfarin

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Table 1 Baseline demographic characteristics of all randomized subjects.

	Treatment sequence	
	Placebo + warfarin → voriconazole + warfarin	Voriconazole + warfarin → placebo + warfarin
Number of subjects	8*	9†
Mean age, years (range)	24 (19–37)	25 (20–29)
Mean weight, kg (range)	74 (62–85)	77 (61–93)
Mean height, cm (range)	180 (168–192)	180 (173–187)

*One subject discontinued during study. †Three subjects discontinued during study.

clearance and confirmation that drug interactions involving terbinafine are unlikely with substrates of CYP isoforms other than CYP2D6 [15].

Many of these clinically significant interactions have been attributed to specific inhibition of the metabolism of the more pharmacologically active (S)-enantiomer of warfarin [16]. These interactions can result in potentiation of the hypoprothrombinaemic effect of warfarin and can frequently result in bleeding complications. Given its propensity to interact with other drugs, it is considered important to establish whether or not any new drug entering clinical practice affects prothrombin time when administered concomitantly with warfarin.

The present study was therefore designed to determine the effects of coadministration of voriconazole (300 mg twice daily for 12 days) and warfarin (single oral dose of 30 mg) on prothrombin time, and to evaluate the safety and tolerability of these drugs when coadministered.

Methods

Study subjects

Following the provision of written informed consent, sufficient numbers of healthy male volunteers to ensure that at least 14 subjects completed the study were screened and enrolled. All subjects were aged 18–45 years, weighed 60–100 kg and were within the permitted body mass range for their height [17].

Volunteers were excluded if there was any evidence of clinically significant disease, allergy, drug sensitivity, or laboratory test abnormalities. A personal or family history of bleeding disorders, a history of peptic ulceration, or an abnormal prothrombin time were also grounds for exclusion. Volunteers were advised not to consume alcohol, methylxanthines including caffeine, or to undertake any strenuous exercise during the 48 h prior to, or during the study. The study protocol was approved in writing by the Clinical Research Ethics Committee, Anatole France Street, Brussels, Belgium.

Study design

This was a double-blind, randomized, placebo-controlled, two-way crossover study with each period

separated by a minimum of 7 days washout. Subjects were randomized to receive either 300 mg voriconazole or placebo twice daily for a total of 12 days. On day 7 of each study period, a single 30-mg oral dose of warfarin was coadministered with the morning dose of voriconazole/placebo. Within each study period, subjects were allocated to one of the following treatment sequences: voriconazole + warfarin followed by voriconazole + placebo or voriconazole + placebo followed by voriconazole + warfarin (Table 1). There was a minimum 7-day washout between each treatment period.

Subjects were admitted to the study unit on the evening prior to first dosing, and were resident for a total of 16 nights throughout the whole study (day 0 and days 6–12 of each study period). Subjects were discharged on the morning of day 13 if their prothrombin time was considered acceptable by the investigator.

Pharmacokinetic sampling

On day 12 of each study period, blood samples (5 ml) for the determination of plasma concentrations of voriconazole were collected in heparinized tubes, predose, and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 h postdose. Samples were centrifuged within 60 min of collection at 1500 g for 10 min at 4 °C, and stored prior to assay in screw-capped polypropylene tubes at –20 °C.

Pharmacodynamic sampling

Blood samples (2 × 2.5 ml) were collected into 3.8% citrate for the determination of prothrombin time at screening, predose on day 1, immediately prewarfarin dose on day 7 and at 4, 8, 12, 24, 36, 48, 60, 72, 96 120 and 144 h post warfarin dose. A 3-ml blood sample was also taken during the study, or at discharge, for determination of CYP2C19 genotype status (Regipharma, Brussels, Belgium).

Safety assessments

Safety assessments were repeated throughout the study, from screening (up to 3 weeks prior to first dose), until follow-up (7–10 days post final dose). Assessments

included physical examinations (supine blood pressure, pulse rate, ophthalmological tests), 12-lead electrocardiograms (ECGs), and routine haematology and urinary analyses.

All adverse events that occurred during treatment, or up to 30 days post final dose, were documented according to their severity, time of onset and duration, and the investigator's assessment of their relationship to treatment. Events involving adverse drug reactions, illnesses with onset during the study, or exacerbations of pre-existing conditions were recorded. Objective test findings that resulted in dosage change or discontinuation were recorded as adverse events. All adverse events were followed up until their sequelae had resolved or stabilized satisfactorily.

Assays

Plasma samples were assayed for voriconazole using a previously validated high-performance liquid chromatography assay [18] (Huntingdon Life Sciences, Huntingdon, UK). Over the calibration range 25–2500 ng mL⁻¹ the interbatch precision and inaccuracy of the method were 2.4–9.1% and 3.8–6.6%, respectively, with a lower limit of quantification of 10 ng mL⁻¹, with imprecision of 1.0% and inaccuracy of –0.1% at this limit. Imprecision is the coefficient of variation of the results in a set of replicate measurements, while inaccuracy is the numerical difference between the mean of a set of replicate measurements and the true value. Prothrombin time was determined within 1 h of sample collection (Biorim, Brussels, Belgium).

Parameter calculations

The maximum observed plasma concentration of voriconazole ($C_{\max}$) and the time to the first occurrence of $C_{\max}$ ($t_{\max}$) were obtained directly from the plasma concentration–time curves. The area under the voriconazole plasma concentration–time curve (AUC_{τ}) within a dosing interval (0–12 h postdose) and the area under the effect curve for prothrombin time over 144 h (AUEC) were determined using the linear trapezoidal rule. The maximum change in prothrombin time was defined as the maximum increase in prothrombin time from baseline (prewarfarin dose on day 7).

Statistical analysis

There were no statistical analyses of the pharmacokinetic data. For the pharmacodynamic data, AUEC and maximum increase from prewarfarin baseline were subject to an ANOVA appropriate for the two-period, two-treatment crossover. The differences between treatment means, stan-

dard errors and 95% confidence intervals for the differences were determined. All analyses and tabulations were performed using SAS/STAT® (SAS Institute Inc., North Carolina, USA) software [19].

A sample size of 14 subjects (seven per sequence group) was considered sufficient to detect a difference of 20% from placebo in prothrombin time AUEC assuming a within-subject standard deviation of 111.9 s·h with probability 0.8 when testing at the 5% level (Pfizer data on file).

Results

Subjects

A total of 17 subjects were screened and subsequently randomized to treatment (Table 1). The demographic characteristics of the subjects who received treatment according to the sequence placebo + warfarin followed by voriconazole + warfarin ($n = 8$) were similar with respect to mean age, weight and height to those of subjects who received treatment according to the sequence voriconazole + warfarin followed by placebo + warfarin ($n = 9$). CYP2C19 genotyping indicated that 14 subjects were homozygous extensive metabolizers, two were heterozygous extensive metabolizers and one was a homozygous poor metabolizer.

A total of 14/16 subjects who received voriconazole + warfarin and 13/15 subjects who received placebo + warfarin completed the study. Two subjects were discontinued after the voriconazole + warfarin treatment period because of prothrombin time prolongation. In addition, two subjects discontinued during the placebo + warfarin treatment period: one due to a protocol violation (use of cannabinoids) having previously completed the voriconazole + warfarin period, while the other withdrew consent after the 12-day placebo + warfarin period. Neither of these discontinuations were considered to be related to study drug.

Pharmacokinetics

For voriconazole, the geometric means of $C_{\max}$ and AUC_{τ} were 3736 ng mL⁻¹ and 25 733 ng·h mL⁻¹, respectively, and the arithmetic mean for $t_{\max}$ was 1.66 h ($n = 16$ throughout). The homozygous poor metabolizer had the highest plasma voriconazole concentration ($C_{\max}$ 8142 ng mL⁻¹) but did not have the maximum prolongation of prothrombin time.

Pharmacodynamics

The mean baseline (prewarfarin) prothrombin time was similar in both the voriconazole (12.9 s) and placebo (12.6 s) periods, suggesting that there was no carry-over effect between treatment periods.

Figure 1 Mean prothrombin time profiles of warfarin for voriconazole and placebo treatment periods.

Coadministration of voriconazole and warfarin resulted in a potentiation of the warfarin-induced increase in prothrombin time. Comparison of adjusted mean AUEC values in the voriconazole (3211 s·h) and placebo (2282 s·h) treatment periods showed a difference of 929 s·h [95% confidence interval (CI) 574, 1283; $P = 0.0002$]. There was also a statistically significant difference in the adjusted mean maximum increase from baseline in prothrombin time between the voriconazole (17 s) and placebo (8 s) treatment periods (95% CI 5, 12; $P = 0.0004$).

From 24 h post warfarin dose, mean prothrombin times were considerably greater in the voriconazole period than in the placebo period. At 144 h post warfarin dose prothrombin time was still increased by a mean of 5.4 s in the voriconazole + warfarin-treated group compared with a mean of 0.6 s in the placebo + warfarin-treated group (Figure 1). Peak prothrombin times occurred at 36 h and 48 h post warfarin dose during the placebo and voriconazole treatment periods, respectively.

Analysis of individual subject profiles for prothrombin time suggested a greater variability between subjects during the voriconazole + warfarin phase, compared with the placebo + warfarin phase. For example, 96 h post warfarin dose, the mean prothrombin times in the two phases were 24.6 s (coefficient of variance 35.8%) and 15.0 s (14.1%), respectively.

Safety

Two subjects were discontinued due to an increase in prothrombin time (Table 2) following coadministration of voriconazole + warfarin during the first period. One subject experienced a moderate increase in prothrombin time from day 8 to 18 (192–432 h post warfarin dose). The second subject was also withdrawn after only one

Table 2 Numbers of subjects reporting adverse events ($n > 1$ in either treatment period).

	Voriconazole + warfarin ($n = 16$)	Placebo + warfarin ($n = 15$)
Total adverse events (treatment-related)	15 (13)	8 (3)
Total causing withdrawal (treatment-related)	2 (2)	0 (0)
Most common adverse events (all causality)		
Abnormal vision	11	1
Headache	4	1
Haemorrhage	4	0
Photophobia	3	0
Asthenia	2	1
Gum haemorrhage	2	1
Prothrombin decreased	2	0
Vasodilatation	2	0
Dry skin	2	0
Rhinitis	0	2

study period because of an increased prothrombin time from day 9 to 13 (216–312 h post warfarin dose), following coadministration of voriconazole + warfarin. This subject was withdrawn as a precautionary safety measure, even though the increase in prothrombin time did not meet the protocol stopping rules of an increase in prothrombin time that was more than three times the baseline. There were no discontinuations due to abnormalities in clinical laboratory test results or physical examinations.

A total of 15/16 subjects in the voriconazole period and 8/15 subjects in the placebo period experienced at least one adverse event (37 and 17 individual events, respectively). These events were considered to be treatment related in 13 and three subjects, respectively (Table 2). The majority of adverse events were mild to moderate in severity. Two severe treatment-related

	Voriconazole + warfarin (n = 16)	Placebo + warfarin (n = 15)
Subjects with visual adverse events	13	2
Enhanced/altered perception*	8	1
Blurred vision*	2	1
Altered colour vision*	3	0
Photophobia*	3	0
Median duration (range)	30 min (15–625)	15.5 min (1–30)
Time until onset, median (range)	15 min (8–710)	454 min (26–882)

*Entries are numbers of events.

adverse events were observed (one photophobia, and one increased prothrombin time), both of which occurred during coadministration of voriconazole and warfarin.

Visual disturbances were reported by 13 subjects in the voriconazole period and two subjects in the placebo period (Table 3). All visual adverse events resolved during the study. Ophthalmological examinations and funduscopy carried out at screening and follow-up showed no abnormalities.

Discussion

The present study demonstrates that voriconazole at a dose of 300 mg twice daily potentiates warfarin-induced prothrombin time prolongation to a degree that might be of clinical significance.

Both the increase in AUEC and the maximum increase from baseline in prothrombin time were significantly greater in the voriconazole + warfarin treatment period compared with the placebo + warfarin treatment period (41% and 100% greater, respectively). Furthermore, these increases in prothrombin time were still present 144 h after voriconazole + warfarin administration, whereas prothrombin times 144 h after placebo + warfarin had almost returned to baseline.

The mechanism for the interaction of voriconazole with warfarin is likely to involve CYP2C9, as this isozyme is estimated to be responsible for approximately 85% of S-warfarin metabolism [20, 21], and is also known to contribute to the metabolism of voriconazole [9]. It has also been reported that genetic polymorphism of CYP2C9 and CYP2C19 may partly account for the large intersubject variability in therapeutic dosages of warfarin [22].

The incidence of CYP2C19 homozygous poor metabolizers in the present study was low (1/17; 6%), which is consistent with previous population studies [23–25]. As expected, this individual had the highest plasma voriconazole concentration; however, the maximum increase in prothrombin time for this subject was not increased compared with other subjects. Although this finding is reas-

suring, further pharmacodynamic studies involving homozygous poor metabolizers may be desirable, to ascertain whether such individuals require different management from patients with other genotypes. Such studies may also help elucidate whether CYP2C genotype was responsible, at least in part, for the increased inter-subject variability in prothrombin time observed in the voriconazole treatment period in the present study.

Although adverse events were more frequently recorded with voriconazole than with placebo, the majority of events consisted of mild to moderate visual disturbances, which have been shown to be short-lived and fully reversible side-effects of voriconazole treatment, experienced by 20–30% of subjects [26, 27]. The incidence of severe adverse events was low, with one case of photophobia and one case of increased prothrombin time recorded during the voriconazole treatment period.

The study protocol required that subjects should be withdrawn if, in the investigator's opinion, they would be at risk if they continued. As a consequence, two (12%) of the subjects were discontinued because of an increased prothrombin time during the voriconazole + warfarin treatment period. As these subjects were subsequently excluded from the data analysis, it should be noted that the overall magnitude of the effect of the coadministration of voriconazole on warfarin-induced prothrombin time prolongation may therefore have been underestimated.

In conclusion, the present study showed that concomitant administration of voriconazole potentiated warfarin-induced prothrombin time prolongation. As a consequence, it is therefore recommended that close monitoring of prothrombin time should be undertaken in patients receiving voriconazole and warfarin concomitantly.

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