Effect of Terbinafine on the Pharmacokinetics of Cyclosporin in Humans

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Cyclosporin is largely metabolized by hepatic cytochrome P450 enzymes, and azole drugs that inhibit cytochrome P450 may precipitate cyclosporin toxicity. The allylamine terbinafine binds to a small subfraction of hepatic cytochrome P450 in type I fashion, and has no effect upon hepatic metabolism of cyclosporin *in vitro*. The purpose of this study was to determine whether oral terbinafine alters the pharmacokinetics of oral cyclosporin *in vivo*.

Twenty male volunteers (age 19–44 years), were randomly allocated to two groups. The first group received three single oral doses of cyclosporin 300 mg at intervals of 21 d. The second and third doses of cyclosporin were preceded by a 6-d course of oral terbinafine 250 mg each morning. A further 250 mg of terbinafine was taken with the second and third doses of cyclosporin. Blood levels of cyclosporin and terbinafine were monitored for 36 h after each dose. The second group received a 7-d course of terbinafine 250 mg each morning. On the seventh day a single dose of cyclosporin 300 mg was taken together with the terbinafine. Blood levels of both cyclosporin and terbinafine were monitored for 36 h. Two further single doses of cyclosporin 300 mg were given at intervals of 2 weeks and the cyclosporin levels again monitored. In both groups each cyclosporin dose was preceded by an 8-h fast.

The mean peak blood concentration of cyclosporin when taken alone was 958 μg/l, and 822 when taken with terbinafine. The mean area under the curve for cyclosporin was 4207 μg/l/h when taken alone and 3665 when taken with terbinafine. The mean absorption half-life for cyclosporin when taken alone was 0.29 h, and 0.33 when taken with terbinafine. The mean time of maximum concentration and elimination half-life of cyclosporin were unaltered by terbinafine. The results suggest that terbinafine is likely to prove a safe systemic anti-fungal treatment for patients who are taking cyclosporin. *Key words: interaction/anti-fungal. J Invest Dermatol 102:740–743, 1994*

Cyclosporin is a cyclic polypeptide immunosuppressive agent extensively used in organ-transplant patients to suppress rejection and graft-versus-host reactions in bone-marrow transplantation, and is also used to treat a wide range of immune-mediated inflammatory conditions [1]. Cyclosporin has a narrow therapeutic range, and has potentially serious side effects. The metabolism of cyclosporin has been shown to be inhibited by azole anti-fungal drugs, both *in vivo* and *in vitro* [2–9]. Terbinafine is a new allylamine class anti-fungal agent used in the treatment of fungal infections of the skin and nails, and has been shown *in vitro* to have no effect upon the hepatic metabolism of cyclosporin [10]. Patients on long-term immunosuppressive therapy have an increased prevalence of colonization with pathogenic fungi [11] and may require systemic anti-fungal therapy. The aim of this study was to determine whether oral terbinafine alters the pharmacokinetics of oral cyclosporin *in vivo*, and thus if it is likely to be safe to prescribe terbinafine in patients receiving concomitant cyclosporin. This was an open, single-center, three-period crossover study in which the period two regimen was repeated in period three. This design was chosen so that any carry-over effects could be tested against the within-subject variability of cyclosporin pharmacokinetics.

Manuscript received June 23, 1993; accepted for publication January 5, 1994.

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Abbreviations: AUC, area under the curve (of the graph of concentration versus time); Cmax, maximum concentration; Tmax, time of maximum concentration.

MATERIALS AND METHODS

Ethical approval was granted by the Joint Ethics Committee of the University of Wales, and the University of Wales College of Medicine. Twenty healthy male volunteers ages from 19 to 44 years, (mean 27.2) were recruited. All were screened to exclude physical illness. A detailed medical and a drug history was taken, and a full physical examination was performed. Samples of blood were taken for a full blood count, liver function tests, and measurement of plasma, urea, creatinine, and electrolytes. The subjects had not received terbinafine or taken any drug known to interfere with the pharmacokinetics of cyclosporin within the previous three months. The subjects were randomly allocated to one of two groups (sequence 1 or sequence 2). The mean height of those randomized to sequence 1 was 177 cm, and of those randomized to sequence 2 was also 177 cm; the mean weights were 75.9 kg and 78.8 kg, respectively.

Those allocated to sequence 1 received three single oral doses of cyclosporin 300 mg at intervals of 21 d. The second and third doses of cyclosporin were preceded by a 6-d course of oral terbinafine 250 mg. A further 250 mg of terbinafine was taken at the same time as the second and third doses of cyclosporin.

Those allocated to sequence 2 received a 7-d course of terbinafine 250 mg first thing each morning. On the seventh day a single dose of cyclosporin 300 mg was taken together with the terbinafine. Two further single doses of cyclosporin 300 mg were taken at intervals of 14 d. In both groups each dose of cyclosporin was preceded by an 8-h fast, and subjects were provided with a standard luncheon 3 h following the dose of cyclosporin and a standard supper in the evening. The study protocol is summarized in Table I.

Samples of blood were collected into glass tubes containing ethylene diaminetetraacetic acid anti-coagulant at 0 h, immediately prior to each dose

0022-202X/94/$07.00

Table I. An Outline of the Study Protocol[a]

	Period 1	Period 2	Period 3
Sequence 1	Cy ⟶	Cy + T ⟶	Cy + T
Sequence 2	Cy + T ⟶	Cy ⟶	Cy

[a] Cy, cyclosporin; T, terbinafine.

of cyclosporin. Further blood samples were taken at 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 24, 28, 32, and 36 h. Whole blood collected for determination of cyclosporin levels was stored at −20°C. Whole blood cyclosporin was measured alone and in combination with cyclosporin metabolites using, respectively, the Cyclo-Trac SP and Cyclo-Trac NS radioimmunoassay kits (INCSstar Corporation, MN). The kits were used according to the manufacturer's instructions until the end of incubation, at which time 500 μl of polylethylene glycol in water was added prior to centrifugation. The calibration standards were prepared using citrated whole blood. The calibration range used was 12.5 to 1600 μg/l and 38.6 to 5288 μg/l for the specific and non-specific assays, respectively. The calibration data were fitted using a four-parameter logistic equation using RIACALC (version 2.57, Pharmacia, Turku, Finland).

Each assay batch contained all of the samples of one subject and four levels of quality control were measured in each assay batch. For the specific assay the nominal concentrations of the four controls were 100, 500, 1000, and 2000 μg/l and the measured coefficient of variation for all was less than 12%. The minimum detectable concentration was estimated as 21 and 29 μg/l for the specific and the non-specific assays, respectively.

Samples of whole blood taken for measurement of terbinafine levels were first centrifuged at 1000 × *g* for 10 min, and the plasma was then drawn and stored in a glass vial at −20°C. The plasma concentration of terbinafine and its N-demethylated metabolite were measured using a high-performance liquid chromatography (HPLC) method. The samples were prepared using the standard method [12], and 100 μl were injected into the HPLC column. Quality control standards received from Sandoz Pharmaceuticals were analyzed to ensure comparibility of the results. The lower limit of detection was 2 ng/ml. The coefficients of variation for terbinafine and its demethylated metabolite were 4.6% and 7.2%, respectively.

For each individual (following each 300-mg dose of cyclosporin) the maximum plasma concentration (Cmax) of cyclosporin and the time to maximum peak concentration (Tmax) were determined. The area under the cyclosporin blood concentration/time curve (AUC) from 0 to 36 h was calculated by the linear trapezoidal rule. The absorption and elimination half-lives were also calculated. The absorption rate for each individual subject was determined using a regression model for all data points up until the Cmax. The slope of the regression line was calculated and from this the absorption rate for that individual according to the formula absorption rate = (log 2)/coefficient of the slope.

A similar calculation was made using points after the Cmax to determine the elimination rate for cyclosporin. The Tmax, Cmax, and AUC (following the seventh daily dose) were also calculated for terbinafine where appropriate.

The pharmacokinetic data was examined using parametric crossover analysis that tested for treatment, period, and carry over effects. The data was then analyzed by treatment (i.e., cyclosporin alone, and cyclosporin plus terbinafine). These data plus the results from the crossover analysis were combined so that the two one-sided tests procedure could be performed to test for bioequivalence between cyclosporin alone and cyclosporin plus terbinafine.

RESULTS

Specific Cyclosporin Assay In sequence 1 the mean Tmax for cyclosporin was 1.8 h when taken alone, and 1.8 and 1.9 h when taken with terbinafine. The mean peak blood concentration of cyclosporin when taken alone was 885 μg/l, and 885 and 772 when taken with terbinafine. The mean AUC for cyclosporin (in sequence 1) were 3755 μg/l/h when cyclosporin was taken alone and 3810 and 3448 when taken with terbinafine. The absorption half-lives for cyclosporin were 0.27 h when taken alone, and 0.34 and 0.32 when taken with terbinafine. The elimination half-lives were 5.35 h when taken alone, and 5.94, and 6.44 when taken with terbinafine.

In sequence 2 the mean Tmax for cyclosporin was 2.0 h when taken with terbinafine and 1.8 and 2.1 when taken alone. The Cmax for cyclosporin was 809 μg/l when cyclosporin was taken with terbinafine, and 979 and 1011 when taken alone. The AUC for cyclosporin was 3736 μg/l/h when taken with terbinafine, and 4591 and 4275 when taken alone. The absorption half-life of cyclosporin was 0.34 h when taken with terbinafine, and 0.28 and 0.31 when taken alone. The elimination half-life of cyclosporin was 5.30 h when taken with terbinafine and 5.74 and 5.49 when taken alone. The mean Tmax, mean Cmax, mean AUC, mean elimination half-life, and mean absorption half-life for cyclosporin for both sequence 1 and sequence 2 are shown in Table II.

Terbinafine Assay In sequence 1 the Cmax for terbinafine was 1500 ng/ml, and 1416 in period 2 and period 3, respectively. The Tmax was 1.01 h and 1.3. The AUCs were 6766 ng/ml/h and 8124. In sequence 2 (period one only) the Cmax for terbinafine was 1120.0 ng/ml and the Tmax was 1.4 h. The AUC was 6244.2

Table II. Pharmacokinetic Data for Cyclosporin by Sequence (Specific Assay)

	Sequence 1	Sequence 2	p Value for Treatment Difference
Tmax (h)			
Period 1	1.81 (1.17–2.45)[a]	2.00 (1.53–2.47)	
Period 2	1.80 (1.14–2.46)	1.81 (1.15–2.47)	NS[b]
Period 3	1.91 (1.24–2.53)	2.11 (1.58–2.64)	
Cmax (μg/l)			
Period 1	885.0 (686–1084)	808.5 (684–933)	
Period 2	884.6 (747–1022)	979.1 (780–1178)	p = 0.03
Period 3	771.7 (515–1028)	1011.1 (878–1144)	
AUC (μg/l/h)			
Period 1	3755.1 (2868–4642)	3735.7 (3263–4209)	
Period 2	3810.3 (3241–4380)	4591.4 (3755–5428)	p = 0.05
Period 3	3447.8 (2608–4288)	4275.2 (3648–4902)	
Absorption $t_{1/2}$ (h)			
Period 1	0.27 (0.21–0.33)	0.34 (0.23–0.45)	
Period 2	0.34 (0.24–0.44)	0.28 (0.22–0.34)	NS
Period 3	0.32 (0.23–0.41)	0.31 (0.27–0.35)	
Elimination $t_{1/2}$ (h)			
Period 1	5.35 (4.88–5.82)	5.30 (4.77–5.83)	
Period 2	5.94 (5.25–6.63)	5.74 (5.07–6.41)	NS
Period 3	6.44 (5.68–7.20)	5.49 (4.84–6.14)	

[a] 95% confidence intervals are given in parentheses.
[b] NS, not significant.

Table III. Pharmacokinetic Data for Terbinafine by Sequence

	Sequence 1	Sequence 2	p Value for Sequence Difference
Tmax (h)			
Period 1		1.4 (1.8–1.0)	
Period 2	1.0 (1.02–0.99)[a]		NS
Period 3	1.3 (1.7–1.0)		
Cmax (ng/ml)			
Period 1		1120 (1158–1082)	
Period 2	1500 (1617–1383)		NS
Period 3	1416 (1557–1276)		
AUC (ng/ml/h)			
Period 1		6244.2 (7459–5029)	
Period 2	6766 (7739–5792)		NS
Period 3	8124 (9216–7032)		

[a] 95% confidence intervals are given in parentheses.

ng/ml/h. No statistical difference was found between the three terbinafine pharmacokinetic profiles. The results of the terbinafine assay are summarized in Table III.

Analysis by Treatment When analyzed by treatment, i.e., cyclosporin alone or cyclosporin plus terbinafine, the mean Tmax for cylosporin was 1.9 h both when taken alone and when taken with terbinafine. The mean Cmax of cyclosporin was 958 when taken alone, and 822 when taken with terbinafine (a mean decrease of 14%). The mean AUC for cyclosporin was 4207 μg/l/h when alone and 3665 when taken with terbinafine. The mean absorption half-life for cyclosporin was 0.29 h when taken alone and 0.33 h when taken with terbinafine. The mean elimination half-life for cyclosporin was 5.5 h when taken alone and 5.9 when taken with terbinafine.

Though the Tmax and the elimination half-lives for the two treatments were found statistically to be bioequivalent, the Cmax, AUC, and absorption half-lives were not. The results of the analysis by treatment is shown in Table IV.

Non-Specific Cyclosporin Assay Statistical analysis of the results of the cyclosporin levels using the non-specific monoclonal assay showed that the Cmax and AUC of cyclosporin were not bioequivalent for the two treatments (i.e., cyclosporin alone or cyclosporin and terbinafine); however the Tmax for both treatments were found to be bioequivalent (as they were for the specific assay). The results of the non-specific assay are shown in Table V.

DISCUSSION

Individuals with impaired cell-mediated immunity are at risk from developing both superficial and systemic fungal infections. Shuttleworth *et al* demonstrated an increased prevalence of colonization with pathogenic fungi in transplant patients on long-term immunosuppresion than normal age- and sex-matched controls [11]. Cyclosporin is a cyclic polypeptide immunosuppressive agent extensively used in transplant patients to suppress rejection and graft-versus-host reactions in bone-marrow transplantation, and also to treat a wide range of autoimmune diseases [1]. Cyclosporin is largely metabolized by hepatic cytochrome P450 enzymes to form its primary hydroxylated and N-demethylated metabolites M17, M1, and M21, which have negligible pharmacologic activity. Drugs that are metabolized by or which induce hepatic cytochrome P450 enzymes may interfere with the metabolism of cyclosporin [1]. Cyclosporin has a narrow therapeutic range, and increased blood levels of cyclosporin may lead to nephrotoxicity with hypertension and hyperkalaemia. Other side effects include gastrointestinal disturbance, hepatotoxicity, hirsutism, acne, gum hypertrophy, and neurotoxicity. Decreased blood levels of cyclosporin in transplant patients may lead to graft rejection, and in patients receiving cyclosporin for other indications lower levels may lead to a decreased therapeutic effect.

The metabolism of cyclosporin has been shown to be inhibited by azole anti-fungal drugs, both *in vivo* and *in vitro* [2–10]. Azoles inhibit a cytochrome P450 enzyme lanosterol 14-demethylase that is involved in fungal ergosterol, and hence fungal cell membrane, bio-synthesis [13,14], and azoles have also been shown to inhibit hepatic cytochrome P-450 III A4, an important enzyme in the metabolism of cyclosporin [15]. The azole drug fluconazole has been shown to be less inhibitory of hepatic cyclosporin metabolism *in vitro* than either ketoconazole or itraconazole, and Kruger *et al* showed no evidence of a significant interaction with cyclosporin in bone-marrow–transplant recipients [16]. However clinically important interactions with cyclosporin have been reported at higher doses of fluconazole [5–7].

Terbinafine is a fungicidal drug of the allylamine class with a broad spectrum antifungal activity and is indicated for fungal infection of the skin and nails caused by *Trichophyton, Microsporum sp.,* and *Epidermophyton floccosum* when oral therapy is considered appropriate. Allylamines inhibit fungal squalene epoxidase, a non-cytochrome P450 enzyme, which is also important in fungal ergosterol synthesis [17]. Although terbinafine is metabolized by hepatic cytochrome P450 it has only a moderate affinity for one relatively small fraction of these enzymes [18,19]. In contrast, azoles have a high affinity for hepatic cytochrome P450 enzymes [20]. Back and Tija demonstrated that terbinafine does not inhibit the metabolism of cyclosporin by human liver microsomes *in vitro* [10].

The results of this study support the results of the *in vivo* investigations that suggested that terbinafine would not cause an increase in the blood concentration of cyclosporin. In fact, however, both

Table IV. Pharmacokinetic Data for Cyclosporin Analyzed by Treatment

	Cyclosporin	Cyclosporin + Terbinafine	Two One-Sided Tests for Bioequivalence
Tmax (h)	1.90 (1.6–2.2)[a]	1.9 (1.6–2.2)	Bioequivalent
Cmax (μg/l)	958.4 (866–1051)	821.6 (728–915)	Not bioequivalent
AUC (μg/l/h)	4207.3 (3788–4627)	3664.6 (3334–3996)	Not bioequivalent
Absorption $t_{1/2}$ (h)	0.29 (0.26–0.32)	0.33 (0.28–0.38)	Not bioequivalent
Elimination $t_{1/2}$ (h)	5.5 (5.16–5.84)	5.9 (5.53–6.27)	Bioequivalent

[a] 95% confidence intervals are given in parentheses.

Table V. Pharmacokinetic Data for Cyclosporin Analyzed by Treatment (Non-Specific Assay)

	Cyclosporin	Cyclosporin + Terbinafine	Two One-Sided Tests for Bioequivalence
Tmax (h)	2.44 (2.73–2.15)[a]	2.31 (2.62–1.99)	Bioequivalent
Cmax (μg/l)	1,468 (1,631–1,304)	1,309 (1,434–1,185)	Not Bioequivalent
AUC (μg/l/h)	10,919.7 (12,157.3–9,682)	9,371.9 (10,350–8,394)	Not Bioequivalent

[a] 95% confidence intervals are given in parentheses.

the AUC and the Cmax for cyclosporin were marginally decreased by the concomitant administration of terbinafine. The absorption half-life of cyclosporin is slightly increased, but the Tmax and elimination half life of cyclosporin remain unchanged. The results of the non-specific cyclosporin assay, which measures the level of cyclosporin as well as its metabolites, when analyzed for bioequivalence using the two-sided test for bioequivalence, were similar to that for the specific assay in that the Tmax for both were bioequivalent, but the Cmax and AUC were not.

There are three possible explanations for this phenomenon. Firstly, it is possible that terbinafine could be acting as an enzyme inducer, increasing the rate of cyclosporin metabolism, but this is unlikely based on the pre-clinical data [10]. Alternatively, terbinafine could decrease the absorption of cyclosporin by some as-yet-unexplained mechanism. However, the most likely explanation is that the differences observed are due to the wide inter- and intra-individual variation of cyclosporin absorption from the current formulation of Sandimmun. Lindholm *et al* have found up to twofold intraindividual variation and more than threefold interindividual variation in the AUC of cyclosporin even when the cyclosporin was given under standard conditions on a weight for weight basis [21]. We believe that this wide intra- and inter-individual variation in cyclosporin absorption explains some of the apparent anomalies seen (Table II). In sequence 1, periods 1 and 2 (respectively, cyclosporin alone, and cyclosporin with terbinifine), the Cmax and AUC for cyclosporin are very similar, but not in periods 2 and 3 (both cyclosporin with terbinafine). In sequence 2 the Cmax and AUC for cyclosporin in periods 2 and 3 (both cyclosporin alone) appear higher rather than lower in the terbinafine phase (period 1) (cyclosporin with terbinafine). However, these differences (between the different periods) were not statistically significant.

The reduction in the AUC for cyclosporin when taken with terbinafine was small, and there was no evidence to suggest that terbinafine would increase the blood levels of cyclosporin. Thus terbinafine is likely to prove a safe systemic anti-fungal treatment for patients who are taking cyclosporin, and the problem of cyclosporin toxicity associated with the use of azoles should be avoided.

We are grateful to Sandoz Pharmaceuticals (UK) Ltd. for sponsoring the costs of this study.

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