Itraconazole solution: higher serum drug concentrations and better clinical response rates than the capsule formulation in acquired immunodeficiency syndrome patients with candidosis

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Abstract

Aims—To compare the serum concentrations of itraconazole and hydroxy-itraconazole after treatment with itraconazole cyclodextrin solution and itraconazole capsules in human immunodeficiency virus (HIV) positive patients with oral candidosis.

Methods—The pharmacokinetics of itraconazole and its active metabolite hydroxy-itraconazole were assessed on days 1 and 7 of therapy in aquired immunodeficiency syndrome (AIDS) patients with oral candidosis taking either itraconazole solution or capsules and the serum concentrations (measured by high performance liquid chromatography) correlated with the clinical response to therapy. In addition, the in vitro susceptibility of *Candida* spp isolates taken from patients at the start of the therapy was assessed.

Results—Nine of 16 patients treated with itraconazole capsules and eight of 15 treated with the solution responded to treatment. Three of the non-responders in each treatment group were infected with isolates resistant to itraconazole in vitro. Although with both preparations there was considerable inter-patient variability in the maximum recorded serum concentrations of itraconazole, they were significantly lower on day 1 and day 7 in those receiving capsules compared with those taking the solution. Patients unresponsive to therapy, but infected with susceptible isolates, had significantly lower concentrations of itraconazole and hydroxy-itraconazole levels on days 1 and 7 than patients responding to treatment. However, patients infected with itraconazole resistant isolates (tested in vitro) failed to respond to treatment despite achieving similar serum concentrations of itraconazole and hydroxy-itraconazole to the responsive patients. For patients with in vitro susceptible isolates a serum itraconazole concentration of < 1000 ng/ml on day 7 was predictive of therapeutic failure (specificity 71%, sensitivity 100%).

Conclusions—Itraconazole cyclodextrin solution achieves higher serum itraconazole and hydroxy-itraconazole concentrations than the capsule formulation in AIDS patients, and this is associated with improved efficacy.

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Oral candidosis unresponsive to azole therapy is a growing problem among human immunodeficiency virus (HIV) seropositive individuals and may be attributed to increased drug metabolism (owing to hepatic enzyme induction by co-prescribed therapies), poor oral bioavailibility, or fungal resistance. Aquired immunodeficiency syndrome (AIDS) related hypochlorhydria has been shown to reduce ketoconazole serum concentrations[1] and itraconazole capsules are similarly dependent upon gastric acid to facilitate absorption.[2] To combat this problem, a cyclodextrin solution of itraconazole has been developed, the absorption of which is less dependent on gastric acidity. The cyclodextrin solution has been found to be effective in AIDS patients with candidosis unresponsive to itraconazole capsules, presumably reflecting improved drug absorption.[3] The success of the solution in patients unresponsive to fluconazole[3] is in keeping with the lack of clinically significant cross resistance between these two azoles in the majority of *Candida* spp isolates from AIDS patients.[4]

We studied a cohort of AIDS patients vulnerable to azole failure because of advanced immunosuppression, prolonged prior azole exposure and, in some, confirmed fluconazole resistance. These patients, all with symptomatic candidosis, were treated with itraconazole capsules or solution, and clinical outcome was correlated with fungal in vitro susceptibility to itraconazole and the drug serum concentrations achieved.

Methods

Thirty one homosexual men with AIDS, CD4 lymphocyte counts < 100 cells/mm³, long histories of prior azole exposure, and pseudomembraneous candidosis (chosen as these characteristics are associated with azole failure) were recruited. Patients were excluded if they were known to be allergic to imidazole antifungals, had severe hepatic or renal impairment, or

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were receiving rifampicin, rifabutin or other hepatic enzyme inducing agents. The study was approved by the local ethics committee.

All patients had pseudomembraneous oral candidosis and provided a mouth swish sample for culture, species identification, and in vitro susceptibility testing. All patients were treated with itraconazole 200 mg every 12 hours for seven days. Sixteen patients were treated with itraconazole coated pellet capsules and 15 with itraconazole cyclodextrin solution (six patients were evaluated on separate occasions with each preparation). Eight patients receiving itraconazole capsules and nine patients receiving the solution underwent pharmacokinetic sampling on the first day of therapy (day 1) with venous samples taken from a peripheral line at 0, 0.5, 1, 2, 4, 8, and 24 hours after the administration of the first 200 mg of itraconazole. Fourteen patients receiving itraconazole capsules and 12 receiving itraconazole solution underwent pharmacokinetic sampling on the final day of treatment (day 7) at the same time points as day 1. Treatment on days 1 and 7 was directly observed in all cases. In 18 cases (eight prescribed capsules and 10 prescribed solution) the subjects were inpatients during the study period and thus therapy was directly observed. Outpatients kept records of dosing times and returned treatment packs at the end of the therapy. In no cases were doses missed or treatment returned unused.

Serum itraconazole and hydroxy-itraconazole concentrations were measured using reversed phase high performance liquid chromatography. The method used has previously been shown to have a lower detection limit of 10 ng/ml with coefficients of variance from 2.2% to 7.8% over a range of drug concentrations from 20 to 1600 ng/ml.[5] The standard curve was linear from 10 to 10 000 ng/ml.

The primary pharmacokinetic outcome measures were serum itraconazole and hydroxy-itraconazole concentrations expressed as daily maximum recorded concentration. Area under the curve calculation was not attempted as there were insufficient sampling time points for this to be accurate.

IN VITRO SUSCEPTIBILITY TESTING
In vitro susceptibility testing was performed as described previously.[6] Clinical specimens were inoculated on to Sabouraud dextrose agar and incubated for 48 hours at 37°C. Three similar colonies were then subcultured on pancreatic casein digest/yeast extract/glucose (CYG) agar (pancreatic casein digest (30 g/l; Merk, Darmstadt, Germany), yeast extract (30 g/l; Difco Laboratories, West Molsey, UK), glucose (60 g/l; BDH Laboratory Supplies, Poole, UK), MOPS (morpholinepropanesulphonic acid; 100 g/l; BDH), Tris (30 g/l pH 7.2; BDH), bacto agar (20 g/l; Difco). These subcultures were used to inoculate lightly 5 ml volumes of dilute CYG broth (1/10), which were incubated for 18–24 hours at 30°C, rotating at 20 rpm to ensure a consistent turbidity. Thus, each isolate was tested in triplicate. The broth cultures were diluted 1/400 into sterile

distilled water to prepare an inoculum of ~ 10^5 colony forming units/ml.

Flat bottomed microtitre plates (ICN Biomedicals Ltd, Oxfordshire, UK) were charged with 20 µl of concentrated CYG broth (100-fold concentration of the dilute CYG broth) mixed with itraconazole (10^{-6} M; 0.7 µg/ml). Inoculum in 200 µl volumes was added to each of these wells. For each colony, three positive control wells (containing inoculum, CYG but no antifungal agent) were also prepared. Negative control wells contained 20 µl of concentrated CYG and 200 µl of sterile distilled water.

The plates were sealed with acetate strips (ICN) and incubated at 37°C for three days, after which the acetate strips were removed and turbidity was measured on a spectrophotometer at a wavelength of 405 nm. The readings were corrected for background absorbance by subtracting the value of the negative control well.

The mean value optical density was calculated for the three positive control wells (containing no antifungal agent) to give a mean positive control growth. The relative growth in each antifungal agent was calculated by dividing the optical density in the medium containing that antifungal by the mean positive control growth of the same isolate and then expressing it as a percentage. As each isolate was tested in triplicate, the mean of these three relative growth values for each drug were then calculated and used in the analysis. At no time was there a difference of > 20% between the three values for either drug or any given isolate.

Definition of clinical resistance
Patients were evaluated on the seventh day of treatment. Patients with persistent signs of candidosis were considered clinically resistant.

Definition of in vitro resistance
Relative growth in itraconazole containing medium exceeding 68% of the growth in control azole free medium has been shown to correlate well with clinical failure of itraconazole solution[4] and was the cut off point chosen to define in vitro itraconazole resistance in the current study.

STATISTICAL ANALYSES
Non-parametric analyses were performed using Wilcoxon's rank sum test for continuous variables and the χ^2 test (with Yates adjustment for small numbers) for binomial variables.

Results
Seventeen patients failed to respond to itraconazole therapy (nine treated with capsules and eight with the solution) including all six (three in each treatment group) who were infected with *Candida* spp isolates resistant to itraconazole in vitro. Response rates did not differ significantly between those treated as inpatients (10 of 18) or outpatients (seven of 13).

PHARMACOKINETICS OF ITRACONAZOLE
With both preparations, there was considerable inter-patient variability in the maximum recorded serum itraconazole and hydroxy-

Table 1   Maximum recorded serum itraconazole and hydroxy-itraconazole concentrations (median and range) on days 1 and 7 of treatment

	Capsule recipients	Solution recipients	Significance*
Itraconazole			
Day 1	180 ng/ml (21–549)	287 ng/ml (47–1419)	p<0.05
Day 7	741 ng/ml (297–1609)	1326 ng/ml (513–2278)	p<0.05
Hydroxy-itraconazole			
Day 1	140 ng/ml (51–308)	195 ng/ml (71–740)	p>0.05
Day 7	1177 ng/ml (354–2276)	1465 ng/ml (661–3574)	p>0.05

*Wilcoxon rank sum test.

Table 2   Maximum recorded serum concentrations (median and range) of itraconazole, hydroxy-itraconazole, and combined levels of both active azoles on days 1 and 7 of treatment

	Patients with isolates susceptible to itraconazole in vitro		Patients with isolates resistant to itraconazole in vitro (all non-responders)*
	Responders	Non-responders	
Day 1			
Itraconazole	302 ng/ml (31–1419)	93 ng/ml (21–388)	376 ng/ml; 741 ng/ml
Hydroxy-itraconazole	218 ng/ml (64–740)	116 ng/ml (51–134)	176 ng/ml; 462 ng/ml
Combined active azole	535 ng/ml (95–1839)	260 ng/ml (76–522)	552 ng/ml; 910 ng/ml
Day 7			
Itraconazole	1185 ng/ml (333–2278)	629 ng/ml (297–917)	1140 ng/ml (863–2141)
Hydroxy-itraconazole	1383 ng/ml (354–3035)	709 ng/ml (565–942)	2667 ng/ml (1177–3574)
Combined active azole	2480 ng/ml (687–4596)	1307 ng/ml (862–1859)	3461 ng/ml (2077–5464)

*On day 1 of therapy, only two patients with itraconazole resistant isolates underwent pharmacokinetic sampling, hence the values obtained for each is presented.

itraconazole concentrations on days 1 and 7 of treatment (tables 1 and 2). The day 1 peak serum concentrations of itraconazole occurred earlier and were significantly higher in those taking solution compared with those taking capsules. Similarly the peak itraconazole concentration on day 7 for patients taking the solution was significantly higher than for those taking capsules. The peak concentrations of hydroxy-itraconazole (the main biologically active metabolite of itraconazole) were also higher (on days 1 and 7) if solution was taken rather than capsules.

PATIENT RESPONSES AND SERUM DRUG
CONCENTRATIONS
Patients with susceptible isolates
The maximum recorded serum concentrations of itraconazole, hydroxy-itraconazole, and total combined active drug on day 1 were significantly higher in those responsive to treatment (with either formulation) than those who failed therapy (p < 0.05 for all three comparisons).

Similarly, the maximum recorded concentrations of itraconazole, hydroxy-itraconazole, or total combined active drug on day 7 were significantly higher in the group who responded, compared with the group who did not respond (p < 0.05 for all three comparisons) (table 2).

None of the day 7 maximum recorded concentrations for the six patients with susceptible isolates who failed therapy exceeded 1000 ng/ml, whereas 10 of the 14 patients who responded to therapy had maximum itraconazole concentrations above this on day 7, giving this threshold a specificity of 71% and a sensitivity of 100% in predicting treatment failure.

A higher proportion of patients treated with itraconazole solution (seven of 12) had day 7 itraconazole serum concentrations above the 1000 ng/ml threshold than patients treated with itraconazole capsules (five of 14), although this difference did not reach statistical

significance (p = 0.065 by Fisher's exact probability test).

Patients infected with itraconazole resistant isolates
None of the five patients infected with itraconazole resistant isolates responded to therapy. However the maximum itraconazole concentrations on day 1 and 7 in these patients fell into the same range as that seen in individuals who had responded to itraconazole and had susceptible isolates.

Discussion
The most important finding of this study was that among patients infected with itraconazole susceptible isolates, the peak serum concentrations achieved with itraconazole on day 1 or day 7 of therapy correlated with the clinical response. Although area under the curve measurements would have been interesting to assess, sampling was performed at insufficient time points to do this accurately. Instead, this study focused on assessing peak serum concentrations shown to occur between two and four hours after dosing, as this would be more amenable to use as a routine clinical tool in patients unresponsive to itraconazole than the collection of a full pharmacokinetic series. A peak itraconazole concentration < 1000 ng/ml on day 7 was a highly sensitive and fairly specific predictor of treatment failure. Finding such suboptimal concentrations might prompt dose escalation or a change to a more reliably absorbed formulation—such as cyclodextrin solution.

It is likely that the considerable variability in peak serum itraconazole concentrations was due to differences in drug absorption. In general, the peak serum concentrations of patients treated with itraconazole solution were considerably greater than for those taking itraconazole capsules. However, they were still below the concentrations achieved with cap-

sules (mean maximum itraconazole concentration day 1, 315 ng/ml; day 7, 501 ng/ml) or solution (mean maximum itraconazole concentration day 1, 306 ng/ml; day 7, 527 ng/ml) in normal volunteers.[7] Thus in HIV seropositive patients failing therapy with the itraconazole capsules, the solution is likely to achieve higher serum concentrations and thus may be effective, as has been confirmed clinically.[3]

Itraconazole solution appears to be the treatment of first choice for patients with fluconazole resistant candidosis, but will be unsuccessful in the minority of patients with isolates crossresistant to itraconazole. Treatment failure at 400 mg/day despite the detection of susceptible isolates in vitro may warrant increasing the dose of solution if the peak serum concentration of itraconazole on day 7 is < 1000 ng/ml.

Thus, for the treatment of fungal infections in patients with advanced HIV disease, the benefits of the improved drug absorption provided by the solution formulation of itraconazole in comparison to capsules, represents a significant advance.

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