Effect of Calcineurin Inhibitors on Posaconazole Blood Levels as Measured by the MVista Microbiological Assay[▽]

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Calcineurin inhibitors may augment the effects of antifungal drugs in microbiological assays. We examined this interaction in a microbiological assay for posaconazole. No effect was observed. However, concurrent or recently discontinued treatment with other antifungal drugs caused false-positive results, emphasizing a limitation of microbiological assays for antifungal drug level measurement.

Posaconazole is a broad-spectrum antifungal agent used for the prevention and treatment of serious fungal infections. Blood levels are highly variable, and low blood levels are associated with treatment failure in aspergillosis (5, 7). These studies suggest that levels of at least 1 µg/ml are desirable for the treatment of aspergillosis and that drug levels should be monitored. Posaconazole levels can be measured by high-pressure liquid chromatography (4), liquid chromatography/mass spectroscopy (1), and microbiological assay (3). High-pressure liquid chromatography and liquid chromatography/mass spectroscopy assays are difficult to develop, require expensive equipment and skilled personnel, and are not widely available. Microbiological assays overcome these limitations but may be affected by coadministration of other drugs with antifungal activity. Calcineurin inhibitors are used to prevent rejection or graft-versus-host disease in transplant patients and often are coadministered with antifungal drugs. They also exhibit antifungal properties (6), which may interfere with monitoring of antifungal blood levels by microbiological assay. To explore these factors, we examined the effects of calcineurin inhibitors on posaconazole levels via microbiological assay.

Posaconazole (lot no. IRQ-PAZ-04-X-25M1-B; Schering-Plough Research Institute, Kenilworth, NJ) was dissolved in dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO) to a concentration of 1.0 mg/ml. Posaconazole standards (20.0, 5.0, 2.5, 1.25, 0.62, and 0.15 µg/ml) and controls (10.0 and 0.31 µg/ml) were made by serially diluting the 1.0-mg/ml dimethyl sulfoxide stock solution in normal human serum (Scantibodies, Santee, CA). A 10× yeast nitrogen base (YNB) broth was made by dissolving 6.7 g of YNB powder (Becton Dickinson and Company, Sparks, MD) and 5.0 g of dextrose (Fisher; D16-500) in a final volume of 100 ml of sterile water. A 2× YNB broth was made by dissolving 6.7 g of YNB powder, 10.0 g of dextrose, and 5.9 g of sodium citrate tribasic dihydrate (Sigma, St. Louis, MO) in a final volume of 500 ml of sterile water and was adjusted to pH 7.0. Difco Noble Agar (2×) was made by

dissolving 2.55 g of Difco Noble Agar (Fisher Scientific, Chicago, IL) in a final volume of 85 ml of sterile water.

For preparation of the plates used in the assay, *Candida kefyr* (ATCC 46764) was suspended in 1× YNB, made by diluting 0.5 ml of 10× YNB with 4.5 ml of sterile water. The suspension was adjusted to 85 to 91% transmittance at a wavelength of 590 nm and then incubated at 37°C until the transmittance was between 65% and 70%, a process that usually required about 5 h. The *C. kefyr* broth (1.5 ml) was mixed into 85 ml of 2× YNB. This solution was then mixed into the 85 ml of 2× Difco Noble Agar and then poured into Corning 245- by 245- by 20-mm plates. After the plates were allowed to solidify for 20 min, 5-mm-diameter wells were bored with a sterile cork borer, and the agar was aspirated out of the plates. The wells were loaded with 50 µl of the desired sample and placed into a 37°C incubator for 14 to 18 h. The plates were read with Mitutoyo Corporation Digimatic calipers. Two measurements were taken for each sample and entered into a plot. A linear regression line of the form $y = b_0 + b_1(x)$, where y is the log₁₀ of the measured diameter, was used to fit the measured standards. For the assay to be considered acceptable, the R^2 value of the regression line must be >0.95, the 10.0-µg/ml control must fall between 8 and 12 µg/ml, and the 0.31-µg/ml control must fall between 0.186 and 0.372 µg/ml. Fluconazole concentrations were determined by gas-liquid chromatography (GLC) at the Fungus Testing Laboratory (San Antonio, TX) (2).

Residual deidentified plasma specimens from 30 transplant patients receiving cyclosporine, tacrolimus, sirolimus, or a combination of tacrolimus and sirolimus were obtained from the clinical laboratory at Clarian Hospital, Indianapolis, IN, in a project approved as an exempt study by the Institutional Review Board. All patients were taking multiple medications, usually mycophenolate and corticosteroids and occasionally antifungal agents. Patients known to be receiving a concurrent antifungal agent were excluded from the analysis. The samples were stored at –80°C upon receipt at MiraVista Diagnostics (Indianapolis, IN). Ten serum specimens were obtained from healthy laboratory personnel.

Five patients were excluded because, after review of the results, they were found in retrospect to have been receiving

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TABLE 1. Posaconazole microbiological assay results in patients receiving cyclosporine, tacrolimus, or sirolimus, but not posaconazole

Specimen	Calcineurin concn ^a (ng/ml)	Posaconazole concn (μg/ml) ^b	Prior antifungal, time interval (concn) ^c
Cyclosporine			
1	64	ND	
2	97	ND	
3	135	ND	
4	151	ND	
5	158	0.61 (R)	Flu, 11 days (4.26)
7 ^d	293	0.35 (R)	Flu, 3 days (3.98)
8 ^e	307	ND (R)	
10 ^e	875	ND (R)	
Tacrolimus			
11	4.2	ND	
12	4.6	<0.15 (R)	Flu, 7 days (2.05)
13	5.1	0.69 (R)	Flu, 6 wk (0.00)
14	5.5	0.76 (R)	Flu, 7 days (0.00)
15	5.9	ND	
18	7.4	ND	
19	7.9	<0.15 (R)	Flu, 7 days (2.21)
20	8.6	ND	
21	9.2	ND	
22	9.2	ND	
24	11.8	ND	
25	11.8	ND	
26	20.7	ND	
Sirolimus			
27	7.1	ND	
28	8.5	ND	
29 ^e	8.9	ND (R)	
30	9.8	ND	

^a Calcineurin levels were determined by the Clarian Hospital laboratory.^b ND, none detected; R, repeat.^c Concentration of fluconazole (Flu) in μg/ml by GLC (Fungus Testing Laboratory, San Antonio, TX).^d Skipped numbers (6, 9, 16, 17, and 23) represent specimens from patients who were excluded because they were taking fluconazole or voriconazole.^e The posaconazole concentration was <0.15 μg/ml when initially tested but undetectable when repeated.

voriconazole ($n = 3$) or fluconazole ($n = 2$). The posaconazole microbiological assay was positive for all five patients at levels ranging from 1.09 μg/ml to >20 μg/ml. For the remaining 25 patients, key findings are shown in Table 1. False-positive results for posaconazole were observed in nine patients, ranging from <0.15 to 0.79 μg/ml. Upon repeat testing, six were reproducibly positive at concentrations ranging from <0.15 to 0.76 μg/ml. Five of these six patients had received fluconazole within 3 to 11 days of the specimen being tested for posaconazole. The six false-positive specimens were tested for fluconazole by GLC, and four gave positive results ranging from 2.05 to 4.26 μg/ml. Because of the false-positive results for the two patients whose blood specimens were negative for fluconazole, 10 specimens from healthy subjects were tested, and none were positive for posaconazole.

To directly test inhibitory activity, normal human serum was spiked with posaconazole and the calcineurin inhibitors to the concentrations specified in Table 2. The mixture contained

TABLE 2. Effect of tacrolimus or cyclosporine on the posaconazole concentration measured by microbiological assay

Calcineurin inhibitor concn (ng/ml)	Zone of inhibition (mm) ^a		
	Posaconazole at 0.5 μg/ml	Posaconazole at 1.5 μg/ml	Posaconazole at 3.0 μg/ml
Tacrolimus			
10	16.5	21.0	23.2
0 ^b	16.5	20.6	23.2
Cyclosporine			
200	15.7	20.6	23.0
0	15.5	20.5	23.3

^a Rounded to the nearest tenth.^b The 0-ng/ml category represents the control with no calcineurin inhibitor. No zone of inhibition was seen with tacrolimus or cyclosporine alone.

equivalent volumes of posaconazole and the calcineurin inhibitor (25 μl of each per well). The calcineurin inhibitors did not produce a zone of inhibition and did not affect the zone size when added to posaconazole (Table 2).

Microbiological assays for antifungal agents are not specific, as inhibition of the growth of the indicator organism may be influenced by any drug that the patient is taking. This limitation is clearly illustrated in the patients who were taking voriconazole or fluconazole at the time of testing. Interestingly, false-positive results were also observed for patients who had recently discontinued fluconazole but in whom fluconazole was still detectable by GLC. Interference caused by concurrent or recently administered antifungal agents, but not calcineurin inhibitors, is a significant limitation of the microbiological method for antifungal drug level measurement.

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