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Bioassay for Miconazole

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A radial diffusion bioassay for miconazole, which employs *Candida stellatoidea* as the indicator organism, is described. Results from three patients treated with the drug are presented.

First described in 1972, miconazole is a β-substituted 1-phenethyl imidazole with broad-spectrum antifungal activity (1, 7). Chemically, it is a white, crystalline substance only sparingly soluble in water (0.03%) and slightly soluble in organic solvents. In vitro it is active against most medically important fungi at concentrations from 0.1 to 10 μg/ml (2, 3, 5, 7). In vivo it has been shown to be protective against *Coccidioides immitis* (3) and curative against *Candida albicans* (7).

Clinical applications of miconazole include topical therapy of dermatophytic infections and vaginal candidosis and oral treatment of gastrointestinal and systemic mycoses (4). The intravenous use of the drug, still in the experimental stage, has been shown to be effective in *Candida* infections and of some potential benefit in other systemic mycoses (6). This report stems from our investigations with the intravenous form of the drug.

The bioassays were performed using a highly susceptible isolate of *Candida stellatoidea* (minimal inhibitory concentration, 0.05 μg/ml) as the indicator organism. Because of the poor solubility of the drug in water, the pharmaceutical preparation of miconazole for intravenous administration was used as standard material rather than pure substance. This preparation consisted of 10 mg of miconazole per ml in a vehicle containing 0.115 mg of polyethoxylated castor oil per ml, 0.5 mg of sodium bisulfate per ml, 1.62 mg of methylparaben per ml, 0.18 mg of propylparaben per ml, and sufficient acetic acid and sodium acetate to adjust the pH to 4.0 ± 0.5 (Janssen R&D, Inc., New Brunswick, N.J.; lot 74LO4/457). A placebo preparation consisting of the vehicle only was shown to be noninhibitory for the indicator organism when tested in a manner identical to that employed in the bioassay procedure. The medium was Sabouraud agar (Difco). Large, rigid plastic plates (23 by 23 cm) were used.

The intravenous preparation of miconazole was first diluted 1:2 with alcohol and then diluted in pooled, normal human serum that previously had been shown to be noninhibitory for the indicator organism. The final concentration in serum was 16 μg/ml. This solution was then serially diluted in the same serum to give concentrations of 8, 4, 2, 1, and 0.5 μg/ml for the standard curve. *C. stellatoidea* MCV 52.10 was harvested from slants of Sabouraud agar and suspended in saline; the density was adjusted to 70% as measured at 570 nm. A 1-ml amount of the suspension was added to 100 ml of molten agar; the seeded agar was mixed and immediately poured into a plate (23 by 23 cm) placed upon a leveling device. Plates were allowed to harden while on the device. A total of 12 stainless-steel antibiotic assay cylinders were placed on each plate in four rows of three cylinders each. Each cylinder received 0.1 ml of diluted drug or of a clinical specimen. Each drug concentration or specimen was tested in duplicate. Controls included a pooled, normal human serum control from the same lot as used for the preparation of the standard curve and pretreatment samples from patients when available. The standard curve and specimen plates were allowed to prediffuse overnight at 4°C before overnight incubation at 30°C. After incubation, zones of inhibition were measured to the nearest 0.1 mm with a dial vernier caliper. Each zone was measured in triplicate, and the average was taken for use in subsequent statistical analyses. Regression analysis was used to derive statistics for the dose response curves, and the estimating equation was used to calculate specimen concentrations of miconazole.

Bioassays for miconazole were performed on five occasions with sera and cerebrospinal fluid specimens from three different patients. These included a case of articular sporotrichosis treated for 30 days, a case of disseminated as-

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TABLE 1. *Five replicate standard dose response curves for miconazole prepared in pooled, normal human serum*

Assay no.	Diam (mm) of zone of inhibition at drug concn (μg/ml; $\log_2$) of:						r	β	α	Y_c (16 μg/ml)	Y_c (1 μg/ml)
	16 (4)	8 (3)	4 (2)	2 (1)	1 (0)	0.5 (−1)					
1	20.8	19.2	17.7	16.5	14.5	12.8	0.998	1.58	14.55	20.87	14.55
2	23.0	20.5	18.33	16.0	13.16	11.5	0.999	2.34	13.57	22.93	13.57
3	23.84	22.17	20.84	19.0	17.0	14.84	0.997	1.78	16.94	24.07	16.94
4	19.83	17.0	16.6	14.67	12.5	10.5	0.989	1.77	12.52	19.62	12.52
5	20.0	18.67	17.16	15.5	13.16	12.33	0.995	1.62	13.71	20.18	13.71
Mean, SD[a]	21.49 ± 1.63	19.51 ± 1.74	18.13 ± 1.47	16.33 ± 1.47	14.06 ± 1.61	12.39 ± 1.45	0.998	1.82	14.26	21.53	14.26

[a] SD, Standard deviation.

pergillosis treated for 4 weeks, and a case of cryptococcosis in a renal transplant recipient treated for 5 weeks. The first two patients were treated intravenously with 10 mg of miconazole per kg every 8 h; the third patient received both intravenous and intraventricular drug. All specimens were stored at −20°C prior to bioassay; sera had been removed from blood specimens prior to storage.

Excellent linearity and correlation were demonstrated between the concentrations of drug and the corresponding diameters of the resulting zones of inhibition (Table 1). In four bioassays r, or the coefficient of correlation, was 0.995 ($P < 0.001$) or better; the lowest value for r was 0.989. Calculated regression lines for the standard dose response curves were essentially parallel in four of the five bioassays (Fig. 1). Statistics for these four lines were similar, with values for β, the regression coefficient or slope, ranging from 1.58 to 1.78. Values for α, or intercept (Y_c when $X = 0$), ranged from 12.52 to 14.55 mm in the same four curves. The one nonparallel curve also demonstrated excellent linearity and correlation ($r = 0.999$); however,

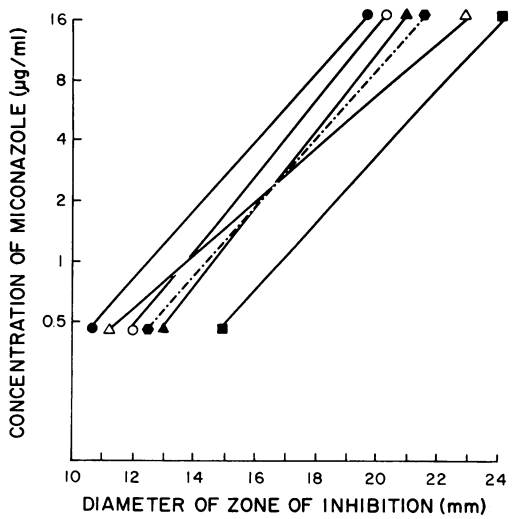

FIG. 1. *Dose response or standard curves obtained for five separate bioassays for miconazole in clinical specimens. The dose response curves were prepared by using an intravenous preparation of the drug diluted in pooled, normal human serum. A highly susceptible isolate of C. stellatoidea was used as the indicator organism. Curves were plotted by using statistics derived by regression analysis. Symbols: ▲, assay no. 1; △, assay no. 2; ■, assay no. 3; ●, assay no. 4; ○, assay no. 5; ⬟, composite standard curve based on averaged zone size values from all five bioassays.*

the slope of this curve, 2.34, differed from the other four, but not significantly ($t = 0.1219$, $P > 0.05$). Excellent correlation was also demonstrated ($r = 0.998$) when a composite regression line based upon mean zone size values of all five curves was derived.

A total of 27 specimens were assayed: sera, 12; cerebrospinal fluids, 6; and ventricular fluids, 9. Eight sera were from the patient with articular sporotrichosis; measurable concentrations of miconazole ranged from 1.41 to 4.35 μg/ml (Table 2). The pretreatment serum was devoid of antifungal activity, and one serum had only a trace amount of less than 0.5 μg/ml. Fourteen specimens from the patient with cryptococcosis were assayed (Table 2). Only three of these contained detectable drug. These included one serum which contained 2.54 μg of drug per ml and two samples of ventricular fluid, obtained immediately after intraventricular administration of 15 mg of miconazole, which contained greater than 120 μg/ml. The results from these two specimens are not to be considered representative of attainable spinal fluid levels. Three sera, including a pretreatment specimen from the patient with disseminated aspergillosis, were assayed; less than 0.5 μg of miconazole per ml was detected in these specimens, including one drawn 30 min after infusion.

Miconazole has been reported to be effective in the treatment of patients with infection due to *C. immitis* (6). In addition, several patients with cryptococcal meningitis are said to have responded to parenteral therapy with this agent (R. Legendre, Janssen R&D, Inc., personal communication). Because of these results and because of its in vitro activity against most fungal pathogens, miconazole may become important clinically. Thus, a reliable bioassay is needed to monitor body fluid levels in terms of bioavailability, therapeutic effectiveness, and potential toxicity. Such a procedure has been described here.

The data presented here have one very important clinical implication. All three fungal isolates from the patients in this study were found to be susceptible to miconazole, being inhibited by 1.56 μg or less of the drug per ml in vitro, yet all three patients failed to respond

TABLE 2. *Results of bioassays for miconazole in 24 clinical specimens from two patients receiving intravenous or intraventricular medication*

Patient	Last miconazole dosage and route[a]	Lapsed time since last dose (h)	Miconazole level (μg/ml) from specimen source:		
			Serum	CSF[b]	Ventricular fluid
Articular sporotrichosis	None	Pretreatment	None		
	10 mg/kg, i.v.	2	4.35	None	
		6.5	1.41	None	
	10 mg/kg, i.v.	7	1.70		
	10 mg/kg, i.v.	9.5	2.00		
	20 mg/kg, p.o.	1	1.70		
		2	1.48		
		8.5	Trace[c]		
Cryptococcal meningitis	10 mg/kg, i.v.	2		None	
	10 mg/kg, i.v.	6.5		None	
	10 mg/kg, i.v.	2	2.54		
	10 mg/kg, i.v.	1.5		None	
	10 mg/kg, i.v.	2			None
	10 mg/kg, i.v.	8			None
	10 mg/kg, i.v.	4.5			None
	10 mg/kg, i.v.	7.5			None
	10 mg/kg, i.v.	3.5		None	None
	10 mg/kg, i.v.	1			None
	15 mg, intraventricularly	5 min			121.8
	10 mg/kg, i.v.	7			None
	15 mg, intraventricularly	5 min			250.3

[a] i.v., Intravenous; p.o., oral.
[b] CSF, Cerebrospinal fluid.
[c] Trace, Less than 0.5 μg/ml, low point of standard curve.

favorably to the drug. These failures may be explained, in part, by our inability to measure significant serum and cerebrospinal fluid levels after apparently adequate dosages in two of the three patients. In the patient with articular sporotrichosis, serum levels, although apparently adequate in terms of the in vitro minimal inhibitory concentration, may have failed to reflect actual drug concentrations at the site of infection. Thus, it is apparent that further data are required regarding distribution, metabolism, and bioavailability of this new antifungal agent.

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