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In Vitro Comparison of Activities of Terbinafine and Itraconazole against *Paracoccidioides brasiliensis*

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In vitro, terbinafine is highly active against a broad spectrum of pathogenic fungi. We evaluated the activities of terbinafine and itraconazole against 31 isolates of *Paracoccidioides brasiliensis*. The tests were conducted by using a broth macrodilution procedure. MICs, in micrograms per milliliter, were as follows: terbinafine, 0.015 to 1.0 (geometric mean, 0.1188); itraconazole, 0.007 to 0.5 (geometric mean, 0.03165). The usual therapy for paracoccidioidomycosis is sulfonamides, amphotericin B, and azole derivatives (ketoconazole, itraconazole, and fluconazole). In comparison to amphotericin B, azole derivatives allow shorter treatment courses, can be administered orally, and are equally effective. Itraconazole has as high efficacy as ketoconazole, but with superior tolerance. It is the current drug of choice for treatment of paracoccidioidomycosis. The data obtained in this study indicate that terbinafine is active against *P. brasiliensis* in vitro and suggest that this allylamine can be considered a new option as drug therapy for paracoccidioidomycosis.

Paracoccidioidomycosis, caused by the dimorphic fungus *Paracoccidioides brasiliensis*, is a systemic human mycosis geographically confined to Latin America (1, 25, 28). In vitro tests of the susceptibility of *P. brasiliensis* to antimycotic drugs have been scarce, and the results have not always been consistent; the discrepancies are attributed to the diversity of techniques employed in such studies (7, 8,10, 11, 22, 28).

Organisms belonging to the kingdom Fungi share eukaryotic characteristics with their human host cells, implying similarities in biochemistry and physiology that limit therapeutics. Therefore, when systemic administration of drugs is required for deep human mycoses, few compounds are sufficiently selective (12). Compounds currently used in the control of infection caused by *P. brasiliensis* include amphotericin B, trimethoprim-sulfamethoxazole, and azole derivatives (26). Unfortunately, amphotericin B has been associated with substantial toxicity, while trimethoprim-sulfamethoxazole has been associated with relapses. Thus, in many clinical situations, the azole derivatives present the best therapeutic option for control of paracoccidioidomycosis (17, 18).

Owing to the fact that itraconazole, among the azoles, permits shorter treatment courses and has been shown to be more effective, we decided to compare this drug with the antifungal agent terbinafine, which operates by interfering with the ergosterol biosynthetic pathway and has shown MICs similar to those of itraconazole for filamentous fungi in in vitro studies (10, 15). Thus, we aim to determine whether terbinafine might have a role in the management of infections caused by *P. brasiliensis*.

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MATERIALS AND METHODS

Fungal strains and culture conditions. Thirty-one isolates of the *P. brasiliensis* yeast form, including clinical, environmental, and animal isolates, were examined in this study. Samples consisted of 28 strains isolated from humans, 1 from penguin feces, 1 from dog food, and 1 from an armadillo. Quality control isolates included Pb-JT-1 (ATCC 90659), Pb-9 (ATCC 36324), and Pb-73 (ATCC 32071). The following characteristics of the isolates are given in Table 1: date of sampling, geographic location, and, when isolates originated from a human source, the clinical form and manifestation, type and location of lesions, and the patient's sex, age, and occupation. Isolates were adapted to a McVeigh-Morton (MVM) chemically defined culture medium with a pH of 7.0 (24), and yeast cells in the exponential phase of growth were obtained by transports at 5- to 7-day intervals and incubation at 35°C (7, 24). The strains are maintained at the Mycology Laboratory, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, in Fava-Netto solid medium (5) by means of subculture at 30- to 40-day intervals and incubation at 35°C.

Susceptibility testing. (i) Antifungal agents. The azole derivative used in this study was itraconazole (Janssen, Beerse, Belgium). Terbinafine was obtained from the Novartis Research Institute (Vienna, Austria). These drugs were obtained from their manufacturers as standard powders, each from a single lot.

(ii) Determination of MICs. The MICs of itraconazole and terbinafine for each strain were determined by using a broth macrodilution procedure according to the work of Shadomy et al. (30).

Stock solutions of itraconazole (1 mg/ml) were freshly prepared in dimethyl sulfoxide (DMSO). Serial twofold dilutions of itraconazole were made by using MVM medium as the diluent to yield final drug concentrations ranging from 4 to 0.007 μg/ml (6). For terbinafine, stock solutions were also freshly prepared in DMSO and further diluted in sterile MVM medium as necessary. A twofold dilution series ranging from 32 to 0.015 μg/ml was employed (16). Drug-free and titrated solubilizing vehicle (DMSO) controls were included. Inocula were determined spectrophotometrically by using a yeast phase suspension in sterile 0.85% saline that gave 69 to 70% transmittance at 520 nm. The inoculum was quantified by yeast cell counts. Yeast cells were collected from the solid medium (MVM) and diluted (1:10) in a counting solution containing 0.9% NaCl, 4% formaldehyde, and 4% Tween 20. The mixture was vortexed to disperse aggregated cells. Counting was performed in a Neubauer chamber. An initial inoculum density of 10^5 cells/ml was obtained (22).

A 0.1-ml aliquot of this suspension was then added to 0.9 ml of MVM broth containing the desired dilution of the drug, giving a final concentration of approximately 10^4 cells/ml (4, 6,22). Strains were grown at 35°C under agitation in a shaker. MICs of itraconazole and terbinafine were defined as the lowest concentration that resulted in a visual turbidity corresponding to 80% inhibition compared with the turbidity produced by the growth control (6). Since there is no

TABLE 1. *P. brasiliensis* isolates

Strain	Yr of isolation	Region and country of isolation	Patient characteristic			Localization or type of lesion in patient, or origin	Clinical form
			Sex	Age (yr)	Occupation		
Pb-73 (ATCC 32071)	1970	Antioquia, Colombia	Unknown	Unknown	Unknown	Unknown	Unknown
Pb-9 (ATCC 36324)	1960	Caracas, Venezuela	Unknown	Unknown	Unknown	Lymph nodes and mucocutaneous lesions	Unknown
Pb-306	1991	Trujillo, Venezuela	Unknown	Unknown	Unknown	Mouth, lung	Unknown
Pb-333	1996	Lima, Peru	Unknown	Unknown	Unknown	Subcutaneous abscess	Unknown
Pb-Tatu		Pará, Brazil				Armadillo	
Pb-Pinguim		Antarctica, Uruguay				Stool of a penguin	
Pb-262		Minas Gerais, Brazil				Dog food	
Pb-JT-1 (ATCC 90659)	1990	Minas Gerais, Brazil					Chronic
Pb-JT-5	1993	Minas Gerais, Brazil	Female	56	Housemaid	Generalized lymphadenopathy	Chronic
Pb-18		São Paulo, Brazil	Unknown	Unknown	Unknown	Unknown	Chronic
Pb-265		São Paulo, Brazil	Unknown	Unknown	Unknown	Unknown	Unknown
Pb-SN		São Paulo, Brazil	Unknown	Unknown	Unknown	Unknown	Chronic
Pb-1087	2000	Rio Grande do Sul, Brazil	Male	60	Retired	Sputum	Chronic
Pb-686	2000	Rio Grande do Sul, Brazil	Male	55	Chicken farmer	Sputum	Chronic
Pb-189	1999	Mato Grosso, Brazil	Male	35	Retailer	Oral mucous membrane	Chronic
Pb-YRJ	1999	Mato Grosso, Brazil	Male	22	Carpenter	Lymph node biopsy	Subacute
Pb-206	1999	Mato Grosso, Brazil	Male	40	Farm worker	Ganglion aspirate	Chronic
Pb-283	1999	Mato Grosso, Brazil	Female	23	Housewife	Ganglion aspirate	Acute
Pb-286	1999	Mato Grosso, Brazil	Male	60	Gardener	Sputum	Chronic
Pb-296	1999	Mato Grosso, Brazil	Male	58	Gardener	Sputum	Chronic
Pb-371	1999	Mato Grosso, Brazil	Male	47	Laborer	Bronchial rinse	Chronic
Pb-375	1999	Mato Grosso, Brazil	Male	42	Electrician	Ganglion aspirate	Chronic
Pb-397	1999	Mato Grosso, Brazil	Male	38	Farm worker	Sputum	Chronic
Pb-694	2000	Mato Grosso, Brazil	Male	47	Farm laborer	Sputum	Chronic
Pb-717	2000	Mato Grosso, Brazil	Male	58	Farm laborer	Ganglion aspirate	Chronic
Pb-769	2000	Roraima, Brazil	Male	48	Farm laborer	Oral mucous membrane	Chronic
Pb-369	2000	Mato Grosso, Brazil	Female	25	Housemaid	Ganglion aspirate	Acute
Pb-84	2001	Mato Grosso, Brazil	Male	59	Mapmaker	Inferior periodontal lesion	Chronic
Pb-61	2001	Mato Grosso, Brazil	Male	39	Driver	Secondary cervical nodule	Chronic
Pb-133	2001	Mato Grosso, Brazil	Male	40	Farm laborer	Ear lobe	Chronic
Pb-57	2001	Mato Grosso, Brazil	Male	42	Farm laborer	Laryngeal mucosa	Chronic

established standard for a terbinafine end point, we arbitrarily selected 80% inhibition as the end point for this antifungal agent in our study (14).

RESULTS AND DISCUSSION

The MIC_{50} and MIC_{90} (MICs at which 50 and 90% of isolates are inhibited, respectively) of both antifungal drugs were determined for all 31 *P. brasiliensis* isolates (Table 2). The geometric mean MIC of itraconazole was 0.03165 mg/ml, and that of terbinafine was 0.1188 mg/ml.

In vitro susceptibilities of *P. brasiliensis* to antifungal drugs have been determined by several investigators (7, 8,11, 14, 23, 29). Some of these previous studies (8, 14, 29) compared susceptibilities in the yeast phase and the mycelial phase of *P. brasiliensis*, finding small differences that were attributed to variations in the lipid composition of the two morphological phases. In the present study we have worked with the yeast form of *P. brasiliensis* for the principal reason that this is the parasitic fungal phase and therefore the target of whatever drug is intended to benefit the human host. Naturally, our results can be compared only with those obtained by other authors with reference to the yeast phase of *P. brasiliensis.*

TABLE 2. In vitro comparison of itraconazole and terbinafine MICs

Agent and strain group	No. of strains tested	MIC (μg/ml)		Geometric mean MIC
		50%	90%	
Itraconazole		0.031	0.125	0.03165
Environmental	2			
Animal	1			
Clinical	25			
Control	3			
Terbinafine		0.125	0.25	0.1188
Environmental	2			
Animal	1			
Clinical	25			
Control	3			

Our MIC data for itraconazole are similar to those reported by others (8, 29), which collectively show that this fungus is extremely sensitive to this drug. No significant difference was observed between the MICs obtained for isolates from patients and those obtained for environmental isolates: Pb-262 (isolated from dry dog food in Minas Gerais, Brazil), Pb-Pinguim (isolated from penguin feces from the Uruguayan Antarctic), and Pb-Tatu (isolated from the intestines of an armadillo captured in the region of Pará, Brazil).

In spite of the facts that itraconazole is currently considered the drug of choice for treatment of paracoccidioidomycosis and that a series of studies have shown its efficacy in clinical cases (17, 18, 31), it is not yet possible to correlate in absolute terms the antifungal activities of the drug in vitro and in vivo. This is principally because of the lack of standardized meth-

odology to determine MICs for dimorphic fungi and the lack of studies that show a parallel between the MIC determined in vitro and therapeutic results obtained during the course of the disease.

Many investigators have used different techniques to determine the MIC. The only standardization for this procedure is the reference method of the NCCLS, adapted primarily for yeasts (19). Nonetheless, this methodology, already in its third version, has not yet been established as the final standard for dimorphic fungi.

In the present study macrodilution in a synthetic medium and preparation of inocula by spectrophotometry were used as proposed by NCCLS M38-P. In both methods the physiological requirements of each tested microorganism are considered in order to allow adequate reading time. Furthermore, the MICs of the azoles in both methods are defined as the lowest concentration resulting in a visual turbidity corresponding to ≥80% inhibition compared with the turbidity of the growth control tube. The MIC for terbinafine was defined as the lowest drug concentration inhibiting 80% of fungal growth, as determined by comparison (as above) to the growth control.

The main differences between the methodology presented here and the modified NCCLS method used in the study by McGinnis et al. with *P. brasiliensis* (14) are the phase of the inoculum (10^4 cells from the mycelial phase in the study by McGinnis et al. as opposed to 10^4 cells of the yeast phase in our study) and the composition of the synthetic medium, including changes in the glucose concentration (0.2% in RPMI 1640 and 1% in MVM medium, respectively). Martinez-Suarez and Rodriguez-Tudela (13) have reported that addition of as much as 2% glucose to culture media helps in the reading of MICs of azole compounds. According to Espinel-Ingroff et al. (3), addition of 2% glucose to RPMI 1640 medium helped in the examination of MIC end points. Perhaps this modification can contribute to the standardization of an adequate methodology for determination of MICs for the study of dimorphic fungi.

The MIC data for terbinafine are homogeneous and low for the isolates tested. Our data agree with the findings of earlier workers who reported that terbinafine has significant antifungal activity against a broad spectrum of fungal organisms (9, 15), including a wide range of filamentous and dimorphic fungi (27).

Because of the variety of test methods employed in these studies, it is difficult to assess the potential clinical relevance of the data. On the other hand, a number of case reports have been published providing MIC data for isolates from various fungal infections successfully treated with terbinafine. These results indicate that favorable in vitro MICs were indeed predictive of clinical success with terbinafine, but of course more data would be required in order to draw any general conclusions (27).

In this study, we demonstrated that terbinafine has potent antifungal activity against *P. brasiliensis* in vitro. Recently, Ollague et al. (20) published a case study of paracoccidioidomycosis successfully treated with terbinafine. If this is confirmed, it will be the first successful treatment of a systemic fungal infection with terbinafine registered in the literature. To date, the promising activity of this drug against dimorphic pathogens has been confirmed clinically only in the case of sporotrichosis (2, 21). Perhaps now its efficacy may be extended to paracoccidioidomycosis.

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