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STUDIES

ANTIFUNGAL ACTIVITY OF ALLYLAMINES AGAINST AGENTS OF EUMYCETOMA

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The antifungal activity of the two allylamines naftifine and terbinafine was investigated against 22 strains of eumycetes isolated from cases of eumycetoma by agar dilution. The Isolates included *Madurella mycetomatis* (4), *M. Grisea* (8), *Pyrenochaeta romeroi* (2), *Exophiala jeanselmei* (2) and *Leptosphaeria tompkinsii* (1) from black grain eumycetomas and *Pseudallescheria boydii* (3) *Acremonium kiliense* (1) and A. *recifei* (1) from pale grain eumycetomas. Terbinafine was more active than naftifine inhibiting 50% (MIC_{50}) and 90% (MIC_{90}) of the black grain eumycetoma agents at 0.5 and 2.5 μg/ml respectively. The MIC_{50s} and MIC_{90s} of naftifine were 1 and 5 μg/ml. For pale grain eumycetoma agents, the MIC range for terbinafine and naftifine were $\leq$0.01 - 100 and 0.1- 100 μg/ml.

Key Words : Allylamines, Eumycetoma

Introduction

Allylamines are the newest group of synthetic antibiotics which act by inhibition of squalene epoxidase in the formation of fungal cell membrane. The two main compounds, naftifine and terbinafine are highly active in vitro against a wide range of fungi.[1-5] Since susceptibility studies of these drugs against the causal agents of eumycetoma are very few, we decided to evaluate the antifungal activity of naftifine and terbinafine (Sandoz Forchungs institute) against the agents of eumycetoma by using a standardised, fragmented inoculum in microcultures. Simultaneous testing was undertaken using the agar dilution method for comparison.

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Materials and Methods

Stock solutions of naftifine and terbinafine were made by initially dissolving 11 mg of the drug in 0.5ml of dimethyl sulfoxide and then adding 4.5 ml of distilled water. Further dilutions were made in distilled water so that 10 μl of each dilution, when added to the microtiter wells, the indicated final concentration was achieved. The final drug concentrations were as follows : 100,20,10, 5, 2.5, 1, 0.5, 0.1, 0.05 and 0.01 μg/ml.

The test organisms were clinical isolates from cases of eumycetoma. Two of the *M. mycetomatis* strains and the *A. kiliense* strain were isolated from patients treated at the Government General Hospital, Madras and they had been deposited at the American Type culture collection (ATCC 62382, 62383, 62384). The *L. tompkinsii* strain was isolated from a patient admitted to the Rajah Mirasdar Hospital, Thanjavur,

amil Nadu and it has been identified as *eptosphaeria* sp., by the Common Jealth Mycological Institute (IML 81764). and *L. tompkinsii* by the merican Type Culture Collection (ATCC 2381). One *M. mycetomatis* strain was btained from the National Institute of Communicable Diseases, New Delhi. The est of the strains were from the Calcutta School of Tropical Medicine, Calcutta, The preparation of the inocula and susceptibility testing by broth microdilution and agar dilution were performed and data obtained as previously described.[6]

Results

The MICs determined by broth dilution are shown in Table I a, b. Terbinafine was more active than naftifine with an MIC value ranging from ≤0.01 - 2.5 μg/ml for black grain eumycetoma

Table I a. Antifungal activity of allylamines in vitro by broth microdilution

Drug	Organism (No. of strains tested)	MIC range (μg/ml)	50%	90%	No. of strains with sensitivity at indicated concentration (μg/ml)									
					≤0.01	0.05	0.1	0.5	1	2.5	5	10	20	100
Nafti-	*M. mycetomatis* (4)	≤0.01-2.5	0.5		1			1	1	1				
fine	*M. grisea* (8)	≤0.01-5	1		1			1	2	2	2			
	P. romeroi (2)	1 - 5							1		1			
	E. jeanselmei (2)	1 - 2.5							1	1				
	L. tompkinsii (1)	≤0.01			1									
	All black grain eumycetoma agents (17)	≤0.01-5	1	5	3			2	5	4	3			
	P. boydii (3)	20-100											1	2
	A. kiliense (1)	0.1					1							
	A. recifei (1)	2.5								1				
	All pale grain mycetoma agents (5) (concluded)	0.1-100	20				1			1			1	2

Table I b. Antifungal activity of allylamines in vitro by broth microdilution

Durg	Organism (No. of strains tested)	MIC range (μg/ml)	50%	90%	No. of strains with sensitivity at indicated concentration (μg/ml).									
					≤0.01	0.05	0.1	0.5	1	2.5	5	10	20	100
Terbi-	*M. mycetomatis* (4)	≤0.01 - 1	0.05		1	1		1	1					
nafine	*M.grisea* (8)	≤0.01-2.5	0.5		1		2	3	1	1				
	P. romeroi (2)	1-2.5							1	1				
	E. jeanselmei (2)	0.1-2.5					1			1				
	L. tompkinsii (1)	≤0.01			1									
	All black grain eumycetoma agents (17)	≤0.01-2.5	0.5	2.5	3	1	3	4	3	3				
	P. boydii (3)	10-100										1		2
	A. kiliense (1)	≤0.01			1									
	A. recifei (1)	1							1					
	All pale grain eumycetoma agents (5)	≤0.01-100	10		1				1			1		2

agents and ≤ 0.01 - 100 μg/ml for pale grain eumycetoma agents whereas the values for nafitifine were ≤ 0.01 - 5 and 0.1 - 100 μg/ml respectively. The MIC values were generally 1 dilution lower by broth dilution method than by agar dilution method.

Comments

Though the allylamines are inhibitory in vitro against a number of pathogenic fungi, only very few strains of the causal agents of eumycetoma have been tested for their susceptibility to these drugs. Our results show that the agents of black grain mycetoma were sensitive in vitro to the allylamines and terbinafine was more active than naftifine.

The MIC_{50s} and MIC_{90s} of terbinafine for agents of black grain eumycetoma were 0.5 and 2.5 μg/ml. When compared with the azoles, ketoconazole, itraconazole, miconazole and econazole, the activity of terbinafine is similar to that of itraconazole which had the same MIC range (≤ 0.01 - 2.5) as well as the value for MIC_{50} and MIC_{90}.[6] The MIC_{50s} and MIC_{90s} of naftifine for black grain eumycetoma agents were 1 and 5 μg/ml.

For the pale grain eumycetoma agents, although the allylamines have inhibited one strain each of *A. kiliense* and *A. recifei* at an MIC of 0.01 and 1 μg/ml of terbinafine and 0.1 and 2.5 μg/ml of naftifine, they are least active with the strains of *P. boydii*. The MIC range of terbinafine and naftifine for the 3 strains of *P. boydii* tested were 10 - 100 and 20 - 100 μg/ml. Shadomy et al[3] have also reported an MIC range of 32 - 64 μg/ml of terbinafine with 50% and 90% inhibition at 64 μg/ml for the 5 isolates of *P. boydii* tested. Their values for naftifine ranged from 16 - 64 with MIC_{50} and MIC_{90} as 16 and 64 μg/ml. [3]Clayton also has reported that *P. boydii* strains were not susceptible to terbinafine.[7]

The in vitro data presented indicate that the allylamines, especially terbinafine is highly active against the agents of black grain eumycetoma. Since terbinafine possesses an highly selective mode of action, can be administered orally and preliminary studies in humans indicate that it is well tolerated, the durg will be very useful for long term therapy of black grain eumycetoma, once it becomes more widely available.

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