ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1978, p. 99-104
0066-4804/78/0014-0099$02.00/0

Vol. 14, No. 1
Printed in U.S.A.

In Vitro Studies with Ambruticin, a New Antifungal Antibiotic

SMITH SHADOMY,* DENNIS M. DIXON, ANA ESPINEL-INGROFF, GERALD E. WAGNER, HUNG PUI YU, AND H. JEAN SHADOMY

Departments of Medicine and Microbiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Received for publication 24 October 1977

The in vitro antifungal inhibitory activities of ambruticin and of various antifungal drugs of choice against 190 fungal pathogens representative of the major human mycoses were compared using a modification of the ICS agar dilution technique. Ambruticin compared favorably with amphotericin B and miconazole when tested against the dimorphic pathogens *Coccidioides immitis, Histoplasma capsulatum,* and *Blastomyces dermatitidis* and against *Aspergillus fumigatus.* Miconazole was the most active compound against *Sporothrix schenckii, Allescheria (Petriellidium) boydii,* and selected dematiaceous fungi, with ambruticin giving minimal inhibitory concentrations from 3- to 74-fold higher. Ambruticin compared unfavorably with amphotericin B and 5-fluorocytosine when tested against *Candida* and *Torulopsis* species. Ambruticin was not as active in vitro as tolnaftate when tested against the three genera of dermatophytic fungi, but compared favorably with miconazole.

With the choice of antifungal chemotherapeutic agents suitable for use in treatment of the mycoses still severely limited, it is essential to examine all potential additions to the clinical armamentarium. Ambruticin (W7783) is a recently described antifungal antibiotic isolated from *Polyangium cellulosum* var. *fulvum* (6a, 7) that has such potential.

A cyclopropyl pyran, ambruticin already has been shown to have antifungal activity in vitro against a variety of filamentous fungal pathogens (7) and also to be both protective and curative in vivo in mice experimentally infected with *Coccidioides immitis* (5, 6). The following study was undertaken to further define the in vitro spectrum of ambruticin and to compare the in vitro antifungal activity of this new compound with the various drugs of choice used in treatment of the several different groups of human mycotic infections (Table 1).

MATERIALS AND METHODS

Drugs. Amphotericin B (E. R. Squibb & Sons; lot no. 22-380-39685-002, activity of 955 μg/ml) and miconazole base (Janssen Research and Development, Inc.; lot no. A14/1) were dissolved in 100% dimethyl sulfoxide. Ambruticin, as the sodium salt (Warner Lambert Research Institute; W7783-1, lot 1, activity of 900 μg/ml), and 5-fluorocytosine (Hoffmann-La-Roche, Inc.; lot no. 21502) were dissolved in sterile physiological saline. Tolnaftate (Schering Corp.; MI no. P-03411) was solubilized by grinding in 95% ethanol. Final stock drug solutions of all five drugs were further diluted to contain 2,560 μg of active material per ml.

Inocula. One hundred and ninety pathogenic fungi were tested (Table 1). These consisted of clinical isolates recovered and identified in our laboratory or cultures from the Center for Disease Control proficiency testing program in Medical Mycology. For the filamentous and dimorphic fungi, inocula were prepared from mycelial-phase cultures grown for 1 month on modified Sabouraud agar slants (Difco) at 25°C. Mature, sporulating cultures were washed with 5 ml of sterile physiological saline, and the resulting suspensions were adjusted so as to contain from 10^4 to 10^5 colony-forming units per ml as confirmed by viable plate counts. For the monomorphic yeasts (*Candida* and *Torulopsis* spp.), cultures were grown on potato dextrose agar (Difco) slants at 25°C for 48 h and were harvested as above except that final inocula were adjusted by nephelometry to contain 10^6 cells per ml.

Media. Selection of media for susceptibility testing was predicated by known requirements of the comparison drugs. With the filamentous and dimorphic fungi, all drugs were incorporated in casein-yeast extract-glucose (CYG) agar (13). For the monomorphic yeasts, ambruticin and miconazole were tested using CYG agar, while 5-FC was tested using solidified, modified yeast nitrogen base agar (8) and amphotericin B was tested using solidified antibiotic medium-3 (Difco; 12).

Susceptibility testing. A modification of the standardized ICS agar dilution technique was employed (1). Drugs were diluted in the specified agar media over a final concentration range of 0.063 to 128 μg/ml. This was done by preparing serial twofold dilutions of the drugs at 10× the final concentrations in broth. Volumes of 2 ml of the resulting drug solutions then

were mixed with 18 ml of sterile, molten agar media and poured into square "Integrid" petri plates (15 by 15 cm, Falcon Plastics). Hardened plates were inoculated using a mechanical replicator delivering inocula of 0.001 to 0.002 ml (Melrose Machine Shop, Woodlyn, Pa.). The plates were incubated at 30°C until growth appeared on drug-free control plates, usually within 2 to 6 days. Minimal inhibitory concentrations (MIC) were defined as the lowest concentration of drug yielding no visible fungal growth.

RESULTS

Results obtained with filamentous and dimorphic agents causing the systemic, opportunistic, and subcutaneous mycoses are shown in Table 2. Data for amphotericin B and miconazole are consistent with previously published results (2, 9, 10). Most isolates of *C. immitis, Histoplasma capsulatum,* and *Blastomyces dermatitidis* were susceptible to all three drugs at concentrations of 1.0 μg/ml or less; the geometric mean MICs (G-MIC) ranged from 0.07 to 1.11 μg/ml. Ten isolates of *C. immitis* were highly susceptible to ambruticin; 90% tested were inhibited by 0.5 μg/ml (G-MIC = 0.41 μg/ml); five of seven isolates of *H. capsulatum* also were inhibited by 0.5 μg of ambruticin per ml (G-MIC = 0.50 μg/ml).

Amphotericin B was the most active drug against *B. dermatitidis* and *Aspergillus fumigatus,* with G-MIC values of 0.11 and 1.20 μg/ml, respectively. Miconazole was the most active drug against *Sporothrix schenckii, Allescheria (Petriellidium) boydii,* and the dematiaceous fungi (*Cladosporium, Fonsecaea,* and *Phialophora* species). G-MIC values of miconazole ranged from 0.56 to 1.24 μg/ml, while those of ambruticin ranged from 2.60 to 41.17 μg/ml. It should be noted that when considering the dematiaceous fungi by genus and species (data not shown), three isolates of *Fonsecaea pedrosoi* were more susceptible to ambruticin (G-MIC = 1.00 μg/ml), whereas isolates of *Phialophora verrucosa, Phialophora jeanselmei,* and the various *Cladosporium* species were more susceptible to miconazole (G-MIC < 1 μg/ml).

Isolates resistant to drug concentrations of 128 μg/ml were encountered with amphotericin B (one isolate of *F. pedrosoi)* and with ambruticin (two isolates each of *Phialophora* species, *A. fumigatus,* and *S. schenckii,* and seven isolates of *A. boydii).* All 16 isolates of *S. schenckii,* 10 of 11 isolates of *A. boydii,* and 11 of the 13 dematiaceous fungi were resistant to clinically significant (2 μg/ml or less) concentrations of amphotericin B. Miconazole was inhibitory for many of these isolates at concentrations of 2 to 4 μg/ml, the probable upper limit of clinical susceptibility to this compound for treatment of systemic and subcutaneous fungal infections (3, 10).

Tolnaftate was by far the most active drug against the majority of the dermatophytic fungi tested (Table 3). The MICs ranged from 0.063 or less to 8 μg/ml, and 82% of the isolates tested were inhibited by 1 μg or less of the drug per ml. *Microsporum canis* was uniquely susceptible to

TABLE 1. *In vitro antifungal comparison of ambruticin and the drugs of choice against 190 isolates of pathogenic fungi*

Organism (no. tested)	Antifungal activity of comparison drugs:				
	Ambruticin	Amphotericin B	Miconazole	5-Fluorocytosine	Tolnaftate
Allescheria (Petriellidium) boydii (11)	X	X	X		
Aspergillus fumigatus (15)	X	X	X		
Blastomyces dermatitidis (6)	X	X	X		
Coccidioides immitis (10)	X	X	X		
Histoplasma capsulatum (7)	X	X	X		
Cladosporium spp. (5)	X	X	X		
Fonsecaea spp. (4)	X	X	X		
Phialophora spp. (4)	X	X	X		
Epidermophyton floccosum (9)	X		X		X
Microsporum audouinii (2)	X		X		X
Microsporum canis (7)	X		X		X
Microsporum gypseum (4)	X		X		X
Trichophyton mentagrophytes (8)	X		X		X
Trichophyton rubrum (18)	X		X		X
Trichophyton tonsurans (16)	X		X		X
Candida albicans (19)	X	X	X	X	
Candida parapsilosis (9)	X	X	X	X	
Candida tropicalis (11)	X	X	X	X	
Torulopsis glabrata (9)	X	X	X	X	

TABLE 2. *In vitro antifungal activities of ambruticin, amphotericin B, and miconazole against 78 isolates of filamentous fungal pathogens*

Organism (no. tested)	Drug[a]	Cumulative % of strains inhibited at drug concn (μg/ml):												G-MIC (μg/ml)
		0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	
Aspergillus fumigatus (15)	Ambruticin					7	27	87					100	5.04
	AMB[a]				13	67	93	100						1.20
	MCZ[b]						27	93	100					3.48
Coccidioides immitis (10)	Ambruticin			60	90			100						0.41
	AMB				100									0.50
	MCZ			20	60	100								0.57
Histoplasma capsulatum (7)	Ambruticin			30	70	100								0.50
	AMB	70	80	100										0.09
	MCZ	80	90	100										0.07
Blastomyces dermatitidis (6)	Ambruticin	33					33					100		1.11
	AMB	33	83	100										0.11
	MCZ	33	50	83	100									0.16
Sporothrix schenckii (16)	Ambruticin							25	69	81			100	12.34
	AMB							13	19	38	44	50	100	41.50
	MCZ					69	100							1.24
Allescheria (Petriellidium) boydii (11)	Ambruticin					9	18	27					100	41.17
	AMB						9		27	36	100			28.21
	MCZ	9		27	55	82	100							0.56
Dematiaceous fungi (13)[b]	Ambruticin	15			38		62	69	77				100	2.60
	AMB				15			23	31		54	77	100	20.89
	MCZ	8	15	31	46	69	77	85			100			1.00

[a] AMB, Amphotericin B; MCZ, miconazole.

[b] *Cladosporium* spp. (five including *Cladosporium carrionii* [two clinical isolates], one proficiency unknown, and one contaminant); three isolates of *F. pedrosoi*; three isolates of *P. jeanselmei*; and one isolate each of *Phialophora verrucosa* and *Phialophora gougerotti*.

tolnaftate, with all seven isolates being susceptible to 0.25 μg or less per ml. These data disagree with those of Weinstein et al. (14) regarding the fungistatic activity of tolnaftate against a variety of dermatophytes as measured in Sabouraud broth, but do agree with those authors' data on *Trichophyton mentagrophytes* and *M. canis*, obtained using an agar dilution procedure employing Sabouraud agar. *Epidermophyton floccosum* was uniquely susceptible to miconazole, with inhibition of all nine isolates by 0.063 μg/ml. Data on miconazole are in agreement with those of Holt (4), who found the nitrate salt to be active against most dermatophytes at concentrations of 10 μg or less per ml.

Ambruticin was more active than miconazole against the *Microsporum* species, with MICs ranging from 1 to 4 μg/ml. However, results with ambruticin and *Trichophyton tonsurans* were variable. Six (38%) of the isolates of *T. tonsurans* tested were resistant to 128 μg/ml, while the remaining 10 isolates were susceptible to 2 μg or less per ml. Three isolates of *Trichophyton rubrum* and two isolates of *E. floccosum* were resistant to 128 μg of ambruticin per ml.

Results obtained from this study clearly showed that ambruticin was much less active than the other agents against most isolates of *Candida* species and *Torulopsis glabrata* (Table 4). Among the *Candida* species, *Candida parapsilosis* was more susceptible, while *Candida tropicalis* and *Candida albicans* were much less so. All nine isolates of *T. glabrata* were resistant to 128 μg of ambruticin per ml.

DISCUSSION

The results with ambruticin and the systemic fungal pathogens, *C. immitis*, *H. capsulatum*, and *B. dermatitidis*, are most encouraging, as the majority of isolates of these three fungi were susceptible to less than 1 μg of this drug per ml. These in vitro results correlate well with results of in vivo experiments with murine coccidioidomycosis in which Levine and Ringel found a high percentage of biological cures, approximately 70%, in mice infected with otherwise lethal doses of *C. immitis* and treated orally with ambruticin (5, 6). Similar results have been obtained in mice experimentally infected with *H. capsulatum* (11).

TABLE 3. *In vitro antifungal activities of ambruticin, miconazole, and tolnaftate against 64 isolates of dermatophytic fungi*

Organism (no. tested)	Drug	Cumulative % of strains inhibited at drug concn (μg/ml):					G-MIC (μg/ml)
		≤0.125	0.25–1.0	2.0–8.0	16–64	≥128	
Microsporum audouinii (2)	Ambruticin			100			2.83
	Miconazole			100			4.00
	Tolnaftate	50		100			0.35
Microsporum canis (7)	Ambruticin		14	100			1.81
	Miconazole		29	100			1.81
	Tolnaftate	86	100				0.10
Microsporum gypseum (4)	Ambruticin			100			2.38
	Miconazole			50	100		9.51
	Tolnaftate	75		100			0.35
Trichophyton mentagrophytes (8)	Ambruticin			88	100		5.66
	Miconazole		12	100			2.59
	Tolnaftate	25	88	100			0.32
Trichophyton rubrum (18)	Ambruticin			72	83	100	8.31 (4.81)[a]
	Miconazole	11	28	94	100		2.52
	Tolnaftate	22	72	100			0.74
Trichophyton tonsurans (16)	Ambruticin		50	62			5.42 (0.82)[a]
	Miconazole		12	94	100		4.36
	Tolnaftate	62	81	100		100	0.30
Epidermophyton floccosum (9)	Ambruticin		78			100	1.47
	Miconazole	100					0.06
	Tolnaftate	100					0.08

[a] G-MIC for susceptible organisms only.

TABLE 4. *In vitro antifungal activities of ambruticin, amphotericin B, miconazole, and 5-fluorocytosine against 48 isolates of clinically important yeasts*

Organism (no. tested)	Drug[a]	Cumulative % of strains inhibited at drug concn (μg/ml):										G-MIC (μg/ml)
		≤0.25	0.5	1	2	4	8	16	32	64	≥128	
Candida albicans (19)	Ambruticin				5	16	32	53	58		100	27.66 (9.07)[b]
	AMB	47	100									0.33
	MCZ				16	58	95	100				4.98
	5-FC	47	58	63		68	74				100	2.11 (0.29)[b]
Candida tropicalis (11)	Ambruticin	9	0	18	0	44	55	64	73	82	100	9.66
	AMB	91								100		0.15
	MCZ						36	64	73	82	100	21.93 (14.81)[b]
	5-FC	91									100	0.22
Candida parapsilosis (9)	Ambruticin	100		56		67	100					2.33
	AMB	11										0.09
	MCZ	78		22	78		89	100				2.16
	5-FC		89						100			0.18
Torulopsis glabrata (9)	Ambruticin										100	>128
	AMB	100										0.13
	MCZ	22	33	44		67	100					1.36
	5-FC	89									100	0.16

[a] AMB, Amphotericin B; MCZ, miconazole; 5-FC, 5-fluorocytosine.
[b] G-MIC for susceptible organisms only.

Infections caused by the dermatophytic fungi are among the most common diseases of humans. This study clearly indicated that ambruticin has in vitro and possibly clinically useful activity against many of the dermatophytic fungi. Although ambruticin was not as active in vitro as tolnaftate, it was comparable to miconazole.

Pharmacological data for this new drug are not yet available. However, peak serum ambruticin levels in mice of 46 μg/ml at 1 h following a 75-mg/kg oral dose of ambruticin have been reported (6a,). Thus, the range of clinically significant in vivo susceptibility for this compound may be as high as 8 to 16 μg/ml. Approximately 70% or more of the isolates of *A. fumigatus, S. schenckii,* and the dematiaceous fungi were susceptible to ambruticin at such concentrations. Ambruticin also was inhibitory for four of seven isolates of *A. boydii* and 13 of 15 isolates of *A. fumigatus* at similar concentrations. Further pharmacologic studies are needed to validate the assumption that 8 to 16 μg/ml represents the upper range of probable in vivo susceptibility to ambruticin.

In this study ambruticin was found to be poorly active or inactive in vitro against monomorphic pathogenic yeasts. In view of the in vivo results reported for ambruticin and dimorphic pathogens, in vivo studies are required to properly evaluate the potential efficacy of this drug in infections caused by yeasts. Further, the observed differences between susceptibilities of yeasts and of filamentous pathogenic fungi to ambruticin, as well as the occurrence of a large number of organisms with apparent primary resistance, suggest an apparently selective mode of action of this new antifungal compound.

ACKNOWLEDGMENTS

These studies were supported by grants from the Warner-Lambert Research Institute and from the Brown-Hazen Program, Research Corp. Funds for purchase of a biological safety cabinet were provided by the Virginia Lung Association.

LITERATURE CITED

1. **Ericsson, H. M., and J. C. Sherris.** 1971. Antibiotic susceptibility testing. Report of an international collaborative study. Acta Pathol. Microbiol. Scand. Sect. B Suppl. **217**:1–68.
2. **Hildick-Smith, G., H. Blank, and H. Sarkany.** 1964. Fungus diseases and their treatment, p. 387–394. Little, Brown & Co., Boston.
3. **Hoeprich, P. D., and E. Goldstein.** 1974. Miconazole therapy for coccidioidomycosis. J. Am. Med. Assoc. **230**:1153–1157.
4. **Holt, R. J.** 1974. Recent developments in antimycotic chemotherapy. Infection **2**:95–107.
5. **Levine, H. B., and S. M. Ringel.** 1977. Efficacy of W7783, a new oral antibiotic in experimental coccidioidomycosis, p. 319–322. *In* L. Ajello (ed.), Coccidioidomycosis: current clinical and diagnostic studies. Am. Col. Chest Physicians. Symposia Specialists Publications, Miami, Fla.
6. **Levine, H. B., and S. M. Ringel.** 1978. Therapeutic properties of ambruticin (W7783) in experimental pulmonary coccidioidomycosis of mice. Chest **73**:202–206.

6a. **Ringel, S. M.** 1978. In vitro and in vivo studies of ambruticin (W7783): new class of antifungal antibiotics. Antimicrob. Agents Chemother. **13**:762– 769.

7. **Ringel, S. M., R. C. Greenough, S. Roemer, D. Conner, A. L. Gutt, B. Blair, G. Kanter, and M. von Strandtmann.** 1977. Ambruticin (W7783), a new antifungal antibiotic. J. Antibiot. **30**:371–375.
8. **Shadomy, S.** 1969. In vitro studies with 5-fluorocytosine. Appl. Microbiol. **17**:871–877.
9. **Shadomy, S., and A. Espinel-Ingroff.** 1974. Susceptibility testing of antifungal agents, p. 659–674. *In* E. H. Lennette, E. H. Spaulding, and J. B. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
10. **Shadomy, S., and L. Paxton.** 1976. In vitro studies with miconazole and miconazole nitrate, p. 171–178. *In* J. D. Williams and A. M. Geddes (ed.), Chemotherapy, vol. 6: Parasites, fungi and viruses. Plenum Press, New York.
11. **Shadomy, S., C. J. Utz, and S. White.** 1978. Studies with ambruticin in murine histoplasmosis. Antimicrob. Agents Chemother. **14**: 95–98.
12. **Utz, C. J., S. White, and S. Shadomy.** 1976. New medium for in vitro susceptibility studies with amphotericin B. Antimicrob. Agents Chemother. **10**:776–777.
13. **Van Den Bossche, H., G. Wellemsens, and J. M. Van Cutsem.** 1975. The action of miconazole on the growth of *Candida albicans.* Sabouraudia **13**:63–67.
14. **Weinstein, M. J., E. M. Oden, and E. Moss.** 1965. Antifungal properties of tolnaftate in vitro and in vivo, p. 595–601. Antimicrob. Agents Chemother. 1964.