Sensitivity of some human pathogenic yeasts and systemic fungi to myxin

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SYNOPSIS Myxin, a relatively new antibacterial and antifungal antibiotic, produced by a species of *Sorangium*, was used to investigate its effectiveness against some yeasts and dimorphic fungi associated with human diseases. Results indicated that the minimal fungicidal concentrations (MFC) of myxin for *Candida albicans*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *Torulopsis glabrata* were 0.39-6.25 µg/ml, and for *C. guilliermondii* and *C. tropicalis* 12.5-25 µg/ml. The MFC for *Blastomyces dermatitidis* and *Sporothrix schenckii* was 0.195 and 6.25 µg/ml, respectively. The MFC for these two systemic fungi for amphotericin B (fungizone) was 0.39-0.78 and 6.25 µg/ml. It seems that myxin is more effective against *B. dermatitidis* than amphotericin B. The isolate of *Coccidioides immitis* was found to be very sensitive to myxin (MFC, 0.78-1.56 µg/ml).

Myxin, a relatively new antibiotic, produced by a species of *Sorangium* (Peterson, Gillespie, and Cook, 1966), has been reported to inhibit the growth of Gram-negative and Gram-positive bacteria, several plant pathogenic and saprophytic fungi, yeasts, and actinomyces (Peterson *et al.*, 1966). Recently, it has been observed that strains of *Sorangium* (= *Lysobacter antibioticus*, Christensen, 1973) inhibited the growth of a strain of *Scopulariopsis brevicaulis*, which was the aetiological agent of deep scopulariopsosis (Sekhon, Willans, and Harvey, 1974). Despite the broad known spectrum of activity of myxin, its effectiveness has not been determined against most fungi which cause disease in man. In order to fill this gap, the present study was conducted to determine the activity of myxin against dimorphic fungi which cause blastomycosis, coccidioidomycosis and sporotrichosis, as well as some human pathogenic yeasts. Comparative studies were also conducted with amphotericin B, the most commonly used antibiotic in cases of systemic fungal infections.

Material and Methods

ORGANISMS

Details relating to their sources are given in table I.

CULTURAL CONDITIONS

The species of yeasts and the dimorphic fungi were

maintained on Phytone-yeast extract agar (BBL) and brain heart infusion agar (BHI, Difco), respectively, at 37°C.

INOCULUM PREPARATION

Inoculum for the yeasts, *Blastomyces dermatitidis* and *Sporothrix schenckii*, was prepared by suspending a loopful of growth (48-168 hours old) in 20 ml of sterile distilled water in a screw-capped bottle. This suspension was shaken vigorously. Spectrophotometric readings taken at 520 nm ranged from 80 to 90% transmission. For *Coccidioides immitis*, 5 ml of sterile distilled water was pipetted into the screw-capped culture slant under the inoculation hood and, after shaking, the suspension was transferred into a sterile bottle that already had 15 ml of sterile distilled water. The transmission reading of this suspension was 95% at 520 nm.

SUSCEPTIBILITY TEST

Susceptibility studies were carried out using yeast nitrogen broth (Difco; Yeast nitrogen base, 6.7 g; 1-asparagine, 1.5 g; dextrose, 10 g; deionized distilled water, 1 litre; sterilized through membrane filter). The procedure used for the test was essentially the same as described in the Center for Disease Control's manual (Haley, 1972). Tube number 1 contained 6 ml of broth and all the other tubes contained 5 ml. The myxin used in this study was obtained from Hoffman-LaRoche Inc, Nutley, New Jersey (RO 7-4488, Lot SM-4, contained 20% water). The amphotericin B (fungizone) was the product of E. R.

PLM No.	Name	Source
293	<i>Candida albicans</i>	Human, 1974, isolated from clinical specimens and identified by the Division of Mycology, Provincial Laboratory of Public Health, University of Alberta, Edmonton, Alberta, using carbon assimilation and fermentation procedure similar to that used by the Center for Disease Control, Atlanta, Georgia, USA
294	" "	
295	<i>C. guilliermondii</i>	
296	" "	
297	" <i>krusei</i> "	
298	" "	
299	" <i>parapsilosis</i> "	
300	" "	
301	" <i>pseudotropicalis</i> "	
302	" <i>tropicalis</i> "	
303	" "	
304	" "	
305	<i>Torulopsis glabrata</i>	As 49A, New York State Department of Health, Albany, NY
306	" "	
307	" "	
218	<i>Blastomyces dermatitidis</i>	As 49B, New York State Department of Health, Albany, NY
217	" "	Human, 1974, Provincial Laboratory of Public Health, Edmonton, Alberta
288	<i>Coccidioides immitis</i>	As B959, from Dr Leo Kaufman, Center for Disease Control, Atlanta, Georgia
253	<i>Sporothrix schenckii</i>	

Table I Sources of the human pathogenic yeasts and systemic fungi

Squibb & Sons Ltd, Montreal. Stock solutions of myxin and amphotericin B were made in acetone and dimethylformamide, respectively (1 mg/ml), and added to the first tube (4 ml) to yield a final volume of 10 ml in this tube. From this tube, after mixing the solution well, an aliquot of 5 ml was withdrawn to prepare further serial dilutions (200-0.195 µg/ml of the broth). With the exception of medium and antibiotic controls (0.195 µg/ml), each tube including solvent controls was inoculated by pipetting 0.05 ml of the previously prepared inoculum suspension. These tubes were then incubated at 30°C until growth was apparent in some of the tubes (48-72 hours for the yeasts, and 96-120 hours for the dimorphic fungi). At the end of the incubation period the tubes were shaken and examined for growth. The first tube showing visible growth, and the next two higher concentrations were used to inoculate Sabouraud dextrose agar (SDA) plates for yeast strains and the BHI slants for dimorphic fungi. In every case transfers from the tube showing visible growth were found to be viable. The lowest concentration from which no growth was obtained was recorded as the minimal fungicidal concentration (MFC).

It was discovered that growth was inhibited in the first two tubes of solvent controls, that is, the tubes corresponding to 400 and 200 µg of antibiotic/ml of the broth, but all other control tubes showed very good growth of the test organisms. This indicates that whatever MFC values have been recorded are due to the antibiotics and not to the solvents.

Results and Discussion

The MFC values obtained for myxin and amphotericin B have been presented in table II. It is clear that myxin is effective in relatively low concentrations against all the organisms included in this investigation. The range of concentration required to inhibit growth of the species of *Candida*, *C. immitis*, and *T. glabrata* is quite narrow. The MFCs for strains of a given species, with a few exceptions, show good correlation. The MFCs for 18 of 19 test organisms are 12.5 µg/ml or less, and 14 out of 19 organisms were inhibited by 6.25 µg/ml or less. Both strains of *B. dermatitidis* were more sensitive to myxin (0.195 µg/ml) than to amphotericin B (0.39-78 µg/ml), whereas the MFCs for these two antibiotics for *S. schenckii* were the same (6.25 µg/ml).

This study indicates that low concentrations of myxin inhibit the growth of yeasts and the dimorphic fungi that cause blastomycosis, coccidioidomycosis, and sporotrichosis. *In vivo* studies are needed to find out whether myxin would be a useful systemic antifungal agent. Such new agents are needed, because amphotericin B and 5-fluorocytosine are sometimes not effective; for example, in a case of candida endophthalmitis, as described by Skidmore and Cockcroft (1974). Also, in this study we have found that myxin yielded very promising results with respect to a strain of *T. glabrata* (PLM 305), which was otherwise found to be resistant to 5-fluorocytosine (2000 µg/ml; unpublished data of the Division of Mycology, Provincial Laboratory of Public Health). This strain was isolated from a patient who later

Organism		MFC ($\mu\text{g/ml}^1$)		
		Myxin	Amphotericin B	5-Fluorocytosine
<i>Candida albicans</i>	293	3-125	(0-39-0-78) ^a	(0-1-100)
"	294	6-25		
" <i>guilliermondii</i>	295	12-5		
"	296	12-5		
" <i>krusei</i>	297	1-56		
"	298	0-78-1-56		
" <i>parapsilosis</i>	299	6-25-12-5	(0-78-3-13)	(100)
"	300	12-5		
" <i>pseudotropicalis</i>	301	0-78		
"	302	1-56		
" <i>tropicalis</i>	303	12-5	(0-39-6-25)	(0-2-50)
"	304	25-0		
<i>Torulopsis glabrata</i>	305	1-56	(0-2-0-78)	2000 ^a
"	306	0-39-0-78		
"	307	0-78		(0-39-1-56)
<i>Blastomyces dermatitidis</i>	218	0-195	0-39-0-78	
"	217	0-195	0-39-0-78	
<i>Coccidioides immitis</i>	288	0-78-1-56	(0-1-0-39)	
<i>Sporothrix schenckii</i>	253	6-25	(0-78-1-56)	
			6-25	
			(3-13-100)	

Table II Sensitivity results of some human pathogenic species of yeasts and systemic fungi to myxin, amphotericin B, and 5-fluorocytosine

¹The MFC is from duplicate determinations.

^aValues in brackets are from the compilation of Shadomy and Espinel-Ingraff (*Manual of Clinical Microbiology*, 2nd ed., American Society for Microbiology, 1974; pp. 569-574). They are for different strains of the same species.

^bThis value is from a single determination.

died with disseminated torulopsosis. Resistance of some strains of *T. glabrata* and some *Candida* species to 5-fluorocytosine was noted by Schönebeck and Ansehn (1973).

Pansy (1968) reported successful use of myxin ointment against skin lesions caused by *Trichophyton mentagrophytes* in guinea pigs. Seven out of 10 artificially induced infections were completely healed. However, no information is available on the absorption or toxicity of myxin from animals or man.

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