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Myxothiazol, a New Antibiotic Interfering with Respiration

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Myxothiazol, a new antibiotic from the myxobacterium *Myxococcus fulvus*, inhibited the growth of many yeasts and fungi at concentrations between 0.01 and 3 μg/ml. It was generally inactive against bacteria. The inhibitory effect was cytostatic. With *Candida albicans*, *Saccharomyces cerevisiae*, and *Mucor hiemalis*, the growth inhibition was neutralized by glucose. Soon after being added to a cell suspension, the compound almost completely blocked oxygen consumption.

The new antibiotic myxothiazol (Fig. 1) is produced by the myxobacterium *Myxococcus fulvus* strain Mx f16. The production and isolation of the compound, some of its biological and physicochemical properties, and its chemical structure have been described recently (2, 6). In this article we report on the mode of action of the antibiotic.

MATERIALS AND METHODS

Organisms. The experimental strains were from the Deutsche Sammlung von Mikroorganismen (DSM) in Göttingen, West Germany; the Centraalbureau voor Schimmelcultures (CBS) in Baarn, The Netherlands; the American Type Culture Collection (ATCC) in Bethesda, Md.; the Institut für Biologie II of the University of Tübingen, (Tü), Tübingen, West Germany; or our own collection (GBF).

MIC. The minimum inhibitory concentrations (MICs) of myxothiazol were determined in liquid cultures by a serial dilution assay with dilution steps of 1:2. The organisms were grown in test tubes containing 3 ml of medium and agitated on a rotary shaker at 160 rpm. The incubation temperature was 30°C; that of *Rhizopus stolonifer* was 25°C. Each culture was inoculated with 10^5 cells per ml. Fungal cultures were started with spores suspended in distilled water. The spores were harvested from a week-old culture on yeast extract agar (3). Most yeasts and fungi were tested in a medium containing 2% peptone (Difco, pH 6.0). *Debaromyces hansenii* and *Nadsonia fulvescens* were tested in the same medium enriched with 1% glycerol; *Saccharomyces cerevisiae* was tested in 0.67% yeast-nitrogen base (Difco)-1% lactic acid (pH 6.0); *Neurospora crassa* and *Schizosaccharomyces pombe* were tested in 2% peptone (Difco)-1% glycerol-0.1% yeast extract (Difco, pH 6.0); and *Ustilago maydis* was tested in 1% Phytone-peptone (BBL Microbiology Systems)-1% glucose (pH 7.0). The bacteria were tested in a medium containing 0.5% peptone from casein, tryptically digested (Merck, Darmstadt, West Germany) plus 0.5% peptone from meat, tryptically digested (Merck) plus 0.05% yeast extract (Difco, pH 7.2).

Inhibition of mycelial growth. The susceptibility of several fungi could only be tested in plate cultures. Agar disks of 6-mm diameter were punched out from the edge of a growing mycelium and placed in the center of plates containing 1% peptone (Difco)-1% glycerol-1% yeast extract (Difco)-1.5% agar (pH 6.0), and myxothiazol in various concentrations. The cultures were incubated at 25°C, and the diameter of the mycelia was measured twice a day.

Effect of myxothiazol on viability. An overnight culture of *Candida albicans* was diluted with prewarmed medium to give a final density of 10^6 cells per ml. Aliquots of 9 ml in 50-ml flasks were shaken for 1 h, and then the antibiotic was added. Samples were taken every 2 h, diluted with distilled water, and plated on yeast extract agar. After 1 day of incubation at 25°C, the colonies were counted.

Glucose antagonism. An overnight culture of *C. albicans* was diluted with fresh medium to a final density of 5×10^5 cells per ml. Aliquots of 6 ml were filled into 10-ml cuvettes of a biophotometer (model Bonet Maury, from Jobin Yvon, Longjumeau, France). Transmission was set at 90%, agitation was set at 100%, and the temperature was set at 30°C. Myxothiazol was added at the beginning of the experiment to all cuvettes but the control. Glucose was added to certain cuvettes at zero time and after 6 h.

Measurement of oxygen uptake. Cells from a log-phase culture of *C. albicans* growing in 0.67% yeast-nitrogen base-1% glucose (pH 6.0) were harvested by centrifugation, washed, and starved by shaking them for 8 to 10 h at 30°C in the same medium without glucose. The cells were then harvested and suspended in fresh glucose-free medium to a final density of 10^8 cells per ml. This suspension was kept in an ice bath until the experiments were started. Oxygen uptake was measured with an oxygen electrode (E 5046 from Radiometer, Krefeld, West Germany) at 30°C. The cell suspension was preincubated with myxothiazol for 5 min at 30°C under aeration and then placed into the 3.4-ml reaction vessel. The reaction was started by adding 50 μl of ethanol.

Application of myxothiazol. The antibiotic was dissolved in freshly distilled dimethyl sulfoxide. In all experiments, dimethyl sulfoxide was added at a con-

centration of 0.83% (vol/vol) and had no inhibitory effect by itself.

RESULTS

Antimicrobial spectrum of myxothiazol. Most of the tested bacteria were not susceptible to the antibiotic. Only a few gram-positive organisms responded at moderately low MICs (Table 1).

All yeasts and fungi were inhibited by myxothiazol at low to very low concentrations (Table 2). The three basidiomycetes could be tested only in plate cultures and were relatively resistant to the antibiotic (Table 3).

Effect of myxothiazol on viability of *C. albicans*. When myxothiazol was added to a log-phase culture, the yeast cells completely stopped multiplication for about 4 h. After this time there was a slow increase in colony-forming units for several hours. This increase was consistently more pronounced with 25.0 μg than with 6.2 μg of myxothiazol per ml (Fig. 2).

Antagonistic effect of glucose. In the presence of glucose, myxothiazol had no inhibitory effect on *C. albicans* (Fig. 3). When glucose was added to a culture which had been blocked before with myxothiazol, growth was resumed after a delay of a few hours. This glucose-myxothiazol antagonism could also be demonstrated with *S. cerevisiae* and *M. hiemalis*.

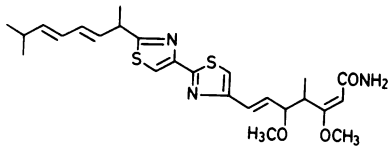

FIG. 1. *Structure of myxothiazol.*

TABLE 1. *MIC of myxothiazol for different bacteria*

Test strain	MIC (μg/ml)
Gram-negative	
Acinetobacter calcoaceticus DSM 30006	>50
Agrobacterium tumefaciens DSM 30150	>50
Alcaligenes eutrophus DSM 531	>50
Escherichia coli DSM 613	>50
Pseudomonas aeruginosa GBF 62	>50
Salmonella typhimurium DSM 50912	>50
Serratia marcescens GBF 61	>50
Gram-positive	
Arthrobacter simplex ATCC 6946	>50
Brevibacterium ammoniagenes DSM 20305	>50
Bacillus megaterium GBF 24	>50
Bacillus subtilis ATCC 6051	>50
Micrococcus luteus GBF 38	3.1
Mycobacterium sp. GBF 3	6.3
Nocardia corallina ATCC 13258	3.1
Staphylococcus aureus GBF 16	>50

TABLE 2. *MICs of myxothiazol for different yeasts and fungi*

Test strain	MIC (μg/ml)
Candida albicans GBF 169	3.1
Debaromyces hansenii DSM 70238	3.1
Hansenula anomala Tü 279	0.4
Nadsonia fulvescens Tü 561	0.2
Nematospora coryli DSM 70350	0.2
Pichia membranaefaciens GBF 31	1.6
Rhodotorula glutinis DSM 70398	1.6
Saccharomyces cerevisiae GBF 36	0.2
Schizosaccharomyces pombe Tü 501	0.1
Torulopsis glabrata DSM 70614	1.6
Mucor lusitanicus CBS 277.49	3.1
M. hiemalis Tü	1.6
Rhizopus stolonifer DSM 63011	0.8
Neurospora crassa 74 A	0.2
Ustilago maydis CBS 132.08	<0.01

TABLE 3. *Inhibition of the radial growth of fungal mycelia in plate cultures by myxothiazol*

Test strain	Concn required to inhibit growth by 50% (μg/ml)	Inhibition by 50 μg/ml (% of control)
Paecilomyces varioti GBF 159	0.1	83
Neurospora crassa 74 A	0.1	100
Polyporus sp. GBF 224	>50.0	30
Polystictus sp. GBF 223	30.0	53
Rhizoctonia solani CBS 177.44	37.0	52

Effect of myxothiazol on the respiration of *C. albicans*. The antibiotic inhibited oxygen consumption by starved yeast cells fairly efficiently. The dose of myxothiazol required to inhibit the respiration of 10^8 cells, suspended in 1 ml, by 50 and 90% was 0.3 and 30 μg, respectively (Fig. 4).

DISCUSSION

The action of the new antibiotic myxothiazol was essentially restricted to eucaryotic cells. In addition to the inhibitory effect for yeasts and fungi as reported in this article and elsewhere (2), high toxicity has also been demonstrated for chicken embryo fibroblasts, ascites cells, and whole animals (mice) (2). Among the bacteria tested, only a few gram-positive organisms responded to the antibiotic. In contrast to a previous statement (2), we found that all yeasts were very susceptible to myxothiazol. The ex-

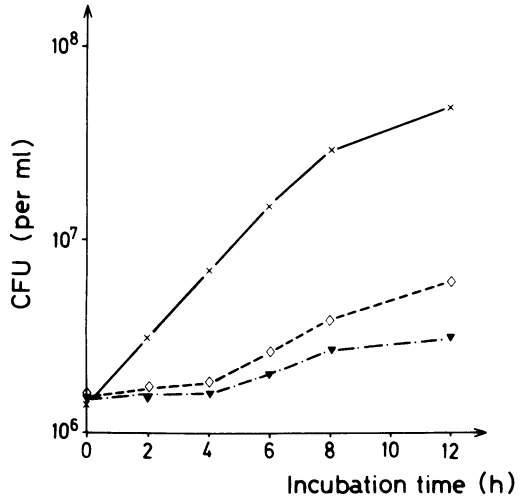

FIG. 2. *Survival of C. albicans in presence of myxothiazol. Symbols: ×, control without the antibiotic; ▼ and ◇, cultures with 6.2 μg (2 times the MIC) and 25.0 μg (8 times the MIC) of myxothiazol per ml, respectively.*

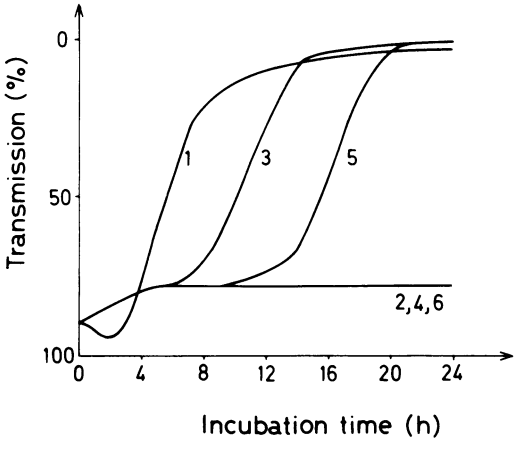

FIG. 3. *Antagonistic effect of glucose to the action of myxothiazol on Candida albicans. 1, Control without the antibiotic and without glucose; 2 to 6, with 12.5 μg of myxothiazol per ml: 2, without further additions; 3, with 1% glucose added at zero time; 4, with water added at zero time; 5, with 1% glucose added after 6 h; and 6, with water added after 6 h.*

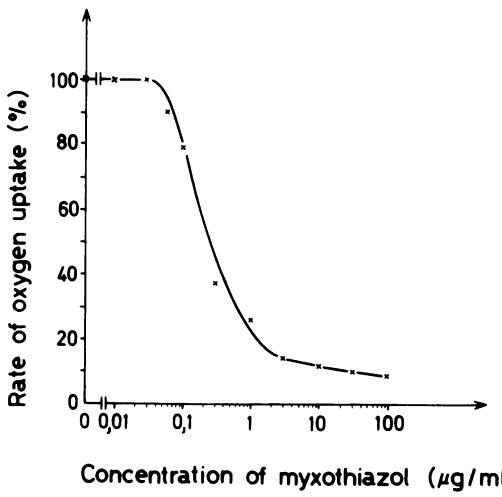

FIG. 4. *Effect of myxothiazol on the rate of oxygen uptake by C. albicans. The density of the experimental cultures was 10^8 cells per ml, and the rate of oxygen uptake by the control culture without the antibiotic was 22.3 nmol of $O_2 \cdot ml^{-1} \cdot min^{-1}$ (100%).*

planation for this discrepancy is that the test media formerly contained glucose, which acts antagonistically to the antibiotic.

The number of *C. albicans* cells able to form colonies upon plating did not decrease over many hours in presence of myxothiazol. In fact, after a log phase of 4 h, the cell number began to slowly increase again, a phenomenon discussed below. Thus, the antibiotic acted only cytostatically. The same has been reported for *M. hiemalis* (2).

The observed antagonism between glucose and myxothiazol was particularly informative. Glucose can be respired as well as fermented by many organisms, and the fact that several fermenting organisms became insusceptible to myxothiazol in the presence of the sugar strongly suggested that the antibiotic interferes with respiration. A glucose antagonism in fermenting organisms is well known for several respiration inhibitors, such as antimycin (1) and mucidin (5). Moreover, an inhibition of oxygen uptake by myxothiazol could be demonstrated experimentally with *C. albicans*, so there is little doubt that our hypothesis is correct. Studies now in progress on mitochondria should show the site in the respiratory chain at which the new antibiotic interferes. A block in the energy metabolism would also fit well with the observation that all macromolecular syntheses break down almost immediately upon addition of the antibiotic, in spite of its effect being gentle enough to be fully reversible (2).

The differences in susceptibility found with different organisms could be due to permeability barriers or to chemical modification of the antibiotic by some of the organisms. Another explanation would be the existence of resistant respiratory pathways or of bypasses of the blocked reaction step. Alternative antimycin- and KCN-

resistant respiratory pathways are well known for several yeasts and fungi, including *C. albicans* (4, 7). Such a mechanism could be responsible for the slow growth in presence of myxothiazol observed with *C. albicans* after a lag phase (Fig. 2).

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LITERATURE CITED

1. **Ahmad, K., H. G. Schneider, and F. M. Strong.** 1950. Studies on the biological action of antimycin A. Arch. Biochem. **28**:281-294.
2. **Gerth, K., H. Irschik, H. Reichenbach, and W. Trowitzsch.** 1980. Myxothiazol, an antibiotic from *Myxococcus fulvus* (Myxobacterales). I. Cultivation, isolation, physico-chemical and biological properties. J. Antibiot. **23**:1474-1479.
3. **Kneifel, H., W. A. König, W. Loeffler, and R. Müller.** 1977. Ophiocordin, an antifungal antibiotic of *Cordyceps ophioglossoides*. Arch. Microbiol. **113**:121-130.
4. **Shepherd, M. G., C. M. Chin, and P. A. Sullivan.** 1978. The alternative respiratory pathway of *Candida albicans*. Arch. Microbiol. **116**:61-67.
5. **Šubík, J., M. Behúň, P. Šmigáň, and V. Musílek.** 1974. Mode of action of mucidin, a new antifungal antibiotic produced by the basidiomycete *Oudemansiella mucida*. Biochim. Biophys. Acta **343**:363-370.
6. **Trowitzsch, W., G. Reifenstahl, V. Wray, and K. Gerth.** 1980. Myxothiazol, an antibiotic from *Myxococcus fulvus* (Myxobacterales). II. Structure elucidation. J. Antibiot. **23**:1480-1490.
7. **Zehentgruber, O., C. B. Kubicek, and M. Röhr.** 1980. Alternative respiration of *Aspergillus niger*. FEMS Microbiol. Lett. **8**:71-74.