ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1972, p. 402-404

Vol. 2, No. 5
Printed in U.S.A.

In Vitro Susceptibility of Isolates of *Aspergillus fumigatus* and *Sporothrix schenckii* to Amphotericin B

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Received for publication 13 June 1972

The in vitro susceptibility of 21 isolates of *Aspergillus fumigatus* and 12 isolates of *Sporothrix schenckii* to amphotericin B was determined. The minimal inhibitory concentration (MIC) for the *A. fumigatus* isolates ranged from 0.14 to 0.6 μg of drug per ml. The mean MIC for the 21 isolates was 0.33 μg per ml. The MIC for the 12 *S. schenckii* isolates ranged from 0.68 to 2.12 μg of drug per ml, with a mean MIC of 1.38 μg per ml.

Reports concerning the susceptibility of *Aspergillus fumigatus* isolates to amphotericin B include that of Gold et al. (6), who found that the tolerance of their *A. fumigatus* isolates increased from 1.9 μg of drug per ml at 24 hr of incubation to 40 μg per ml at 48 hr. Their inoculum consisted of both mycelium and spores, the effects of which will be discussed in more detail later. Ikemoto (7) determined amphotericin B susceptibilities for isolates obtained from a patient with a pulmonary fungus ball. He found the minimal inhibitory concentration (MIC) of amphotericin B for the pretreatment isolate to be 15 μg per ml; this increased to 30 μg per ml for an isolate obtained during treatment, and further increased to 500 μg per ml in a posttreatment isolate. Clayton (3) studied isolates of *A. fumigatus* from infected eyes and arrived at an MIC of 6 μg of amphotericin B per ml. Mohr et al. (10) found that only one of the three isolates that they studied was inhibited by as little as 5 μg of amphotericin B per ml. In contrast, Shadomy (12) studied three isolates of *A. fumigatus* which were inhibited by 0.39 μg of amphotericin per ml. In general, these studies have involved only a very few isolates and have included a wide variety of methods.

The literature concerning the minimal inhibitory concentrations of amphotericin B for *Sporothrix schenckii* is scanty, probably because most patients with lymphocutaneous sporotrichosis are successfully treated with iodides. Pulmonary and disseminated sporotrichosis are usually treated with iodides, or a combination of iodides and amphotericin B, followed by surgery. One paper that does cite some values concerning the susceptibility of *S. schenckii* to amphotericin B is that of Gold et al. (6). Another is that of Shadomy (12), who reported that the MIC ranged from 6.25 to 12.5 μg of drug per ml for a group of five isolates.

Studies of the susceptibility of pathogenic fungi to amphotericin B have also been reported by Bennett (1), Shadomy et al. (13), Marks (9), and Otčenášek and Hubálek (11). There is considerable information concerning the susceptibility of various isolates of *Candida* spp. and *Saccharomyces cerevisiae* because of their use in bioassay procedures.

The purpose of this study was to compare, by means of a simple, reproducible technique, the in vitro susceptibilities of a number of clinical isolates of *A. fumigatus* and *S. schenckii* to amphotericin B.

MATERIALS AND METHODS

The assay technique used was modified from that devised by Shadomy (12). For each test, the contents of a fresh bottle containing 50 mg of a commercial preparation of amphotericin B were suspended in 10 ml of sterile distilled water, and an appropriate amount of this suspension was diluted 1:50 in brain heart infusion broth. This broth was used to make 1:2 serial dilutions in screw-cap test tubes (15 by 150 mm) containing 3-ml quantities of brain heart infusion broth. This process was repeated through 11 tubes. The 12th tube received no drug mixture and served as the growth control for each series. To each tube, 3 ml of Sabhi agar was then added. Final drug concentrations were 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.2, 0.1, and 0.05 μg of drug per ml.

A. fumigatus inocula were prepared by washing spores from the surface of 7-day Czapek agar slant cultures with sterile 0.85% saline containing a small amount of Tween 80. Spore suspensions were homogenized with a Vortex mixer and then adjusted to the

density of a no. 1 McFarland standard with sterile distilled water. A 0.1-ml portion of inoculum was carefully added to the surface of the medium in each tube with a micropipetter. *S. schenckii* inocula were prepared in the same way except that the isolates were grown on modified Sabouraud agar (4) and were harvested with sterile water containing no Tween 80. All tubes were kept in the dark after the drug was added except during inoculation, and all were incubated in the dark. The cultures were incubated at 22 C for 48 hr; they were then examined and the results were recorded. At least three replicates were done for each isolate and the results were averaged. The MIC was considered to be the lowest concentration of the drug causing complete inhibition of growth of a given isolate as determined by visual observation of the absence of mycelia on the medium.

The cultures tested were all isolated from clinical materials from patients with aspergillosis or sporotrichosis. The time interval after initial isolation of the different isolates varied, but was not considered to be a factor since all of the cultures had been kept frozen until they were lyophilized. All cultures had been lyophilized prior to testing, but were morphologically typical when they were revived.

RESULTS

The 21 isolates of *A. fumigatus* tested were inhibited over a range of 0.14 to 0.60 μg of amphotericin B per ml. The mean MIC was 0.33 μg per ml and the median was 0.34 μg per ml. All were susceptible to concentrations of amphotericin B achievable in serum, as determined by Bindschadler and Bennett (2), Fields et al. (5), and Louria (8).

The 12 isolates of *S. schenckii* tested were found to be considerably more resistant to amphotericin B than any of the *A. fumigatus* isolates. They were inhibited by a range of 0.68 to 2.12 μg of drug per ml. The mean MIC for the 12 isolates was 1.38 μg per ml, or over four times that for the *A. fumigatus* isolates. The median value was 1.23 μg per ml.

DISCUSSION

As our studies progressed, it became apparent that the technique used caused less variability in the results than was caused by the type and condition of the inoculum and the length of the incubation period. Our results correlated well with those of others when these factors were controlled.

Warr and Roper (14) and Clayton (3) emphasized that the age of the culture, the size of inoculum used on the test medium, and the make-up of the inoculum all profoundly influence the results. A mycelial inoculum gives a much higher MIC value (6, 14) than does a conidial suspension containing only small amounts of mycelium. Warr and Roper (14) observed that the MIC of amphotericin B varied widely with *A. nidulans* unless they used conidial suspensions that were relatively free from mycelium. We found the same to be true of *A. fumigatus*, but, in general, this fungus lends itself better to the preparation of uniform inocula than does *A. nidulans*. We found it necessary to use a small amount of Tween 80 to achieve uniform suspension of the spores, but after the suspension was carried through to the final dilution there was essentially no detergent left to influence permeability of the spores. Most of the conidiophores and stray bits of mycelium were diluted out also, so that our inoculum consisted almost entirely of conidia suspended in distilled water.

S. schenckii spore suspensions were much easier to prepare because their sympodulospores are very easily dislodged, and the smooth conidia wet with no difficulty. Lyophilized cultures of this fungus sporulated less vigorously than the primary isolates, but we could usually obtain sufficiently heavy spore suspensions from 1-week-old cultures.

The MIC values for our *A. fumigatus* isolates were generally lower than those reported in the literature. This may be due to a number of factors, the most important of which may be the length of the incubation period. Amphotericin B is not stable in vitro, and is fungistatic rather than fungicidal at low concentrations; thus, the MIC increases appreciably with increased incubation time. Because of this, we made a point of reading the tests as soon as there was confluent growth on the drug-free control media (48 hr for these two fungi). One reason that these fungi were selected for study is that both grow rapidly and results could be obtained before there was a serious loss of drug potency. If the test cultures of either fungus were held for up to 1 week, the final MIC values were usually 4- to 10-fold higher than at 48 hr. This increase in MIC values is compatible with the findings of Gold et al. (6) and Mohr et al. (10).

Our *S. schenckii* isolates were considerably more resistant to amphotericin B than the *A. fumigatus* isolates studied. Even so, our MIC values were considerably lower than those reported by Shadomy (12). This difference is probably due, in part, to the lower incubation temperature and shorter incubation period. As previously stated, the results are very much influenced by a variety of factors. As many as possible of these must be standardized if results from various laboratories are to be compared.

The techniques used in this study provide a simple, rapid means of determining relative amphotericin B susceptibilities of fast-growing fungi. The applicability of these results will depend on a critical comparison of these suscepti-

bilities and the corresponding clinical responses of a large number of patients.

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