EFFECTS OF THE ANTIFUNGAL PEPTIDE Ro 2-7758 ON MORPHOLOGY OF MUCOR CORYMBIFERA

GLEN R. GALE

The Veterans Administration Hospital, and the Departments of Physiology and Pharmacology and of Pathology, Duke University Medical Center, Durham, North Carolina

Received for publication 16 November 1962

ABSTRACT

GALE, GLEN R. (Veterans Administration Hospital and Duke University Medical Center, Durham, N.C.). Effects of the antifungal peptide Ro 2-7758 on morphology of Mucor corymbifera. J. Bacteriol. 85:833—837. 1963.—Hyphae of Mucor corymbifera grown in the presence of the antifungal peptide, Ro 2-7758, were examined in an electron microscope. The predominant change, compared with control hyphae, was a marked thickening of the cell wall, with apparent maintenance of normal structure. Cell walls of control hyphae were from 0.03 to 0.11 μ in thickness; drug-treated walls varied from 0.22 to over 0.75 μ in thickness. The possible sites and mechanisms of action, as well as the significance of a thickened cell wall in antibiosis, are discussed.

The isolation from a streptomyecete of an antibiotic with high in vivo activity against certain systemic mycoses coupled with relatively low mammalian toxicity was reported recently (Grunberg, Berger, and Titzworth, 1961; Emmons, 1961; Utz, Andriole, and Emmons, 1961; Andriole, Utz, and Sabesin, 1961). This substance, designated Ro 2-7758 (formerly X-5079C), is unique among currently known antifungal agents in that it is a peptide rather than a polypeptide compound, and has a narrow in vitro antimicrobial spectrum.

In the course of studies concerning the mechanism of antifungal action of Ro 2-7758, a rather peculiar type of inhibition of some of the more rapidly growing fungi on agar plates was noted. Instead of the customary zone of absolute inhibition surrounding a drug-impregnated filter paper strip, as is observed with highly active antimicrobial agents, a limited initial growth of the test organism occurred up to the strip impregnated with Ro 2-7758 at concentrations up to 1,000 times that found by Emmons and Piggott (1961) in sera of patients undergoing therapy with the drug. After 24 to 30 hr, however, growth adjacent to the strip ceased, while that at the edges of the plate continued. Such an inhibitory pattern prompted the electron microscopic comparison of inhibited and uninhibited hyphae reported here.

MATERIALS AND METHODS

Of all the more rapidly growing fungi tested, inhibition was most evident with a strain of Mucor corymbifera (Duke Fungus Registry No. 2002); consequently it was used in all experiments. Solutions of Ro 2-7758 in distilled water (1.0 mg/ml) were sterilized by passage through a Swiny filter (Becton, Dickinson and Co., Rutherford, N.J.). Sterile strips of Whatman no. 1 filter paper were impregnated with the drug solutions and placed on Sabouraud agar plates seeded with a heavy spore suspension of the test organism. For microscopy, small areas of growth from inhibited and uninhibited areas were removed at 1, 2, or 3 days, fixed in 1.5% aqueous KMN04 for 3 hr at 0 C, dehydrated in a graded ethanol series, and embedded in methacrylate (20% methyl and 80% butyl monomers) with ultraviolet polymerization. Sections were cut on a Porter-Blum microtome and examined in an RCA EMU3-F electron microscope, using 100 kv of accelerating voltage through a 30-μ objective aperture.

RESULTS

The most striking difference between hyphae taken from inhibited and uninhibited areas was in the cell wall. Whereas walls of control hyphae were quite thin, measuring from 0.03 to 0.11 μ in thickness, walls of hyphae exposed to the drug ranged from 0.22 to over 0.75 μ in thickness. Figure 1 shows longitudinal sections of control hyphae with walls approximately 0.06 μ in thickness. Hyphae taken from the inhibited zone are shown in Fig. 2.
FIG. 1. Sections of hyphae of Mucor corymbifera grown without Ro 2-7758, showing nuclei (N), mitochondria (M), vacuoles (V), cell walls (CW), and apparent intracytoplasmic membranes (ICM).
FIG. 2. Sections of hyphae of Mucor corymbiferum grown in the presence of Ro 2-7758. Abbreviations same as for Fig. 1.
The comparative morphology of the cell wall was the same whether specimens for fixation were taken at 1, 2, or 3 days; that is, even during the earlier period of incubation when no inhibited zone was evident adjacent to the drug-impregnated filter paper, the electron microscope revealed the same difference in wall thickness as was found at 3 days.

Comparison between intracytoplasmic structures of inhibited and uninhibited hyphae presents problems of interpretation. Mitochondria and vacuoles were obvious in most of the inhibited preparations; nuclei were present but frequently differed from nuclei of control specimens in being slightly more vacuolated. However, Thyagarajan, Conti, and Naylor (1962) found similar low-density areas in nuclei of *Rhodotorula glutinis* which had not been exposed to any agent other than growth medium prior to fixation. Considering the possible effects of a thickened cell wall on passage of permanganate fixative, alcohol, and methacrylate monomers into the cell, no conclusions should be drawn concerning the intracellular structure of inhibited specimens.

Figure 3 shows a section of cell wall from inhibited and uninhibited areas photographed at a higher initial magnification. The control wall is approximately 0.1 \( \mu \) in thickness, while the drug-treated wall is approximately 0.6 \( \mu \). No meaningful structural differences between the two, other than size, can be observed.

**Discussion**

In the case of the effects of penicillin on cell walls of penicillin-sensitive bacteria (Murray, Francombe, and Mayall, 1959), or the dissolution of the cell wall of *Micrococcus lysodeikticus* by lysozyme, there is no problem in reconciling the observed biochemical and morphological events with death of the microorganisms. Such is not the case with the data reported here. So far as can be determined, no previous electron microscopic studies have revealed this peculiar change as a result of drug action. Although Blank, Taplin, and Roth (1960) noted that griseofulvin seemed to cause loss of integrity of cell walls of *Trichophyton rubrum*, thickening of the wall was not a consistent finding, and when it did occur was accompanied by a peculiar lamellation of the wall. So far as can be determined, structure of the walls of *M. corymbifera* grown in the presence of Ro 2-7758 differ from walls of untreated hyphae only in thickness and not in structure.

Two points of interest remain to be clarified: first, the cytological site at which the change in cell wall is brought about and the mechanism by which it occurs, and, second, the possible significance of a thickened cell wall in antibiosis.

Regarding the former, there can be little doubt that cell-wall synthesis must occur at the surface of the cytoplasmic membrane. Synthesis of amino sugar polymers intracellularly with subsequent passage of these high-molecular-weight substances out of the cell seems quite unlikely in view of present concepts of membrane transport and permeability. More data are necessary to determine whether the thickened cell wall is a result of actual stimulation of wall synthesis per se or a result of alteration of a genetic mechanism by which a balance of wall
EFFECTS OF RO 2-7758 ON MUCOR MORPHOLOGY

Synthesis and cytoplasmic synthesis is normally maintained.

As concerns the significance of the thickened wall in antibiosis, it is of course not yet known whether this factor is primary or secondary. However, if wall thickening is a primary event mediated through either of the mechanisms discussed above, the resulting barrier to passage of nutrient materials into the cell and passage of metabolic end products from the cell may be sufficient to account for the observed antibiosis.

ACKNOWLEDGMENTS

This work was aided by grant RG-9389 from the U.S. Public Health Service. The Ro 2-7758 was furnished by John J. Pepper of Hoffmann-La Roche, Inc., Nutley, N.J. The author is grateful to Helen H. McLain for her careful technical assistance. Leonard Hart, Chief of the Medical Illustration Service at the Durham Veterans Hospital, and Jack Evans gave valuable photographic assistance.

LITERATURE CITED