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Susceptibility of Yeast-Like Fungi to a New Antifungal Agent, LY 121019

Die Empfindlichkeit hefeartiger Pilze für das neue Antimyzetikum LY 121019

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Summary: LY 121019, a new antifungal antibiotic agent, was tested for activity against 200 clinical isolates of *Candida* and other yeast-like fungi. LY 121019 had its greatest inhibitory effect on *C. albicans*, and *C. tropicalis*. *C. glabrata* and most other *Candida* species were not as sensitive. *Cryptococcus* and other yeast-like fungi, with the exception of a few strains, were not susceptible to LY 121019.

Zusammenfassung: LY 121019, ein neues Antimyzetikum, wurde auf seine Aktivität gegen 200 klinische Isolate von *Candida* und anderen, hefeähnlichen Pilzen untersucht. LY 121019 hat seine größte Hemmwirkung gegen *C. albicans* und *C. tropicalis*. *C. glabrata* und die meisten anderen *Candida*-Arten waren nicht so empfindlich. *Cryptococcus* und andere, hefeähnliche Pilze waren, mit Ausnahme weniger Stämme, nicht für LY 121019 empfindlich.

Introduction

LY 121019, an analog of the polypeptide antifungal antibiotic echinocandin B, appears to possess antifungal activity primarily against *Candida* species (2, 3, 5, 6). Its antifungal action probably results from inhibition of the biosynthesis of β-1,3-glucan, causing severe damage to the cell wall of yeasts (2). The aim of this study was to investigate the inhibitory and fungicidal effects of LY 121019 on clinical isolates of *Candida* species as well as other yeast-like fungi.

Materials and Methods

Fungi

Two hundred isolates of *Candida* and other yeast-like fungi maintained in our laboratory were examined. These organisms were from two sources: isolates from patients with fungemia, meningitis, and similar deep-seated infections and isolates from patients participating in a study of fungal colonization. Organisms from overnight slant cultures on Sabouraud glucose agar, (Difco, Inc., Detroit, MI.) were suspended in 0.9% saline to a concentration of 2×10^7 CFU/ml (0.55 optical density reading at 660 nm in a spectrophotometer). A dilution of 1:2000 in Sabouraud glucose broth, pH 5.5, (Difco,

Inc.), gave a final inoculum of 1×10^4 CFU/ml.

Antifungal agent

LY 121019, (Lilly Research Laboratories, Indianapolis, Indiana) was solubilized in 50% ethanol at a concentration of 1000 µg/ml. A further dilution of 1:12.5 in Sabouraud glucose broth gave a final concentration of 80 µg/ml.

Determination of minimum inhibitory concentration (MIC)

Microtiter plates with 96 U bottom wells (Flow Laboratories, Inc., McLean VA), were used. Sabouraud's glucose broth (100 µl) was pipetted into each well. The solution containing 80 µg/ml LY 121019 (100 µl) was added to the first well of each row. Serial two-fold dilutions were made across the plate using a Costar octapette (Costar, Inc., Cambridge, MA). The final well received no drug, serving as a positive growth control. The concentration of drug ranged from 0.04 µg/ml to 40 µg/ml. Each fungal isolate (100 µl) was added to each of the 12 wells in a row at a concentration of 1×10^4 CFU/ml.

The plates were incubated at 30°C for 24 to 48h. The MIC was determined as the lowest concentration showing no visible turbidity using a microtiter plate reader. The MIC_{50} was the concentration of drug which inhibited 50% of the isolates and the MIC_{90} was that concentration of drug which inhibited 90% of the isolates.

Determination of minimum fungicidal concentration (MFC)

After reading the MIC for each organism, a 10µl sample was taken from those wells which showed no turbidity and plated on Sabouraud's glucose agar. After incubation at 30°C for 24h, the MFC was read as the lowest concentration of drug in which only 1 or no fungal colonies persisted. The MFC_{50} was the concentration of drug which killed 50% of the isolates while the MFC_{90} was that concentration of drug which killed 90% of the isolates.

Results

The MIC_{50} and MIC_{90} for 74 strains of *C. albicans* were 0.31 µg/ml and 0.62 µg/ml, respectively, with a range from 0.08 µg/ml to >40 µg/ml (Table 1). Only 2 organisms had MIC's >0.62; both of these isolates had MIC's of >40 µg/ml. Most *C. tropicalis* were susceptible, but the MIC_{90} was >40 µg/ml. As noted in Table 1, other species of *Candida* were generally less susceptible to LY 121019 than *C. albicans* and *C. tropicalis*.

The MFC_{50} and MFC_{90} for all strains of *Candida* were high (Table 1). LY 121019 was fungicidal at 2.5 µg/ml or less for 31% of *C. albicans* and 37% of *C. tropicalis*. In those strains of *C. albicans* and *C. tropicalis* in which the MFC was low (0.625–2.5 µg/ml), there was a paradoxical effect or Eagle phenomenon noted on the MFC plates. Although the organism was killed by fairly low concentrations of drug, growth occurred again at the highest concentrations of LY 121019.

The sensitivity of *Cryptococcus* and other yeast-like fungi to LY 121019 is shown in Table 2. Except for 2 strains with MIC's of 5 µg/ml, *Cr. neoformans* was resistant to LY 121019. The few strains of *Cr. laurentii* tested were susceptible to LY 121019. The MIC's for all other yeast-like fungi were >40 µg/ml.

Discussion

This study was carried out to evaluate the spectrum of activity of LY 121019 against a variety of clinical yeast isolates. LY 121019 was most active against *C. albicans* and *C. tropicalis*, the organisms which cause most episodes of disseminated candidosis (4). Although some strains of other species were susceptible to LY 121019, most were resistant. These findings are similar to those

Table 1: Susceptibility of *Candida* to LY 121019

Organisms	Number	MIC (μg/ml) Range	MIC_{50}	MIC_{90}	MFC (μg/ml) Range	MFC_{50}	MFC_{90}
Candida albicans	74	0.08–>40	0.31	0.62	0.31–>40	>40	>40
Candida glabrata	21	5—10	5	10	5–>40	10	20
Candida parapsilosis	20	0.16–>40	20	>40	0.16–>40	40	>40
Candida tropicalis	19	0.16–>40	0.62	40	0.31–>40	20	>40
Candida krusei	9	0.08–10	5	5	0.16–20	10	20
Candida pseudotropicalis	2	20	–	–	>40	–	–
Candida guilliermondii	1	0.31	–	–	>40	–	–
Candida stellatoidea	1	0.31	–	–	0.31	–	–

Table 2: Susceptibility of *Cryptococcus* and other Yeast-Like Fungi to LY 121019

Organisms	Number	MIC (μg/ml) Range	MIC_{50}	MIC_{90}	MFC (μg/ml) Range	MFC_{50}	MFC_{90}
Cryptococcus neoformans	34	5–>40	>40	>40	10–>40	>40	>40
Cryptococcus albidus	2	>40	–	–	>40	–	–
Cryptococcus laurentii	2	0.31–0.62	–	–	>40	–	–
Saccharomyces cerevisae	8	5–>40	>40	>40	20–>40	>40	>40
Rhodotorula rubra	4	>40	>40	>40	>40	>40	>40
Rhodotorula glutinis	1	>40	–	–	>40	–	–
Trichosporon beigelii	1	>40	–	–	>40	–	–
Geotrichum species	1	>40	–	–	>40	–	–

noted by others (2, 3, 5, 6). Levels of >10 μg/ml can be achieved in vivo in experimental animals; if similar concentrations are achieved in humans, LY 121019 might be clinically useful in treating infections due to *C. albicans* and *C. tropicalis*.

Even though the MIC's for most *C. albicans* and *C. tropicalis* were low, for many of these strains LY 121019 was not fungicidal, a finding different from that of Hobbs et al. (3). Additionally, those organisms which had MIC's and MFC's in the range of 0.31 μg/ml to 1.25 μg/ml showed the Eagle effect, with recrudescent growth at high drug concentrations (1). This phenomenon has been noted before by some authors (3, 6) but not by others (2). The relevance of the Eagle effect, described most often with beta-lactam antibiotics, to clinical treatment of infection is unknown.

Yeast-like organisms other than *Candida*, with rare exceptions, were resistant to LY 121019. Thus, it would appear that LY 121019 may prove to be a useful drug in treating certain specific types of yeast infections, but it will not be useful for all such infections.

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Congress-Calendar Kongreßkalender

September 1–3, 1988, Cannes:
8. CIRD-Symposium „Advances in Skin Pharmacology". Hauptthemen: „Pharmacology of Retinoids in the Skin", „Treatment of Skin Diseases", „Prevention and Treatment of Skin Damage", „New Active Chemical Entities and Potential Therapies". Auskunft: Dr. B. Shroot, Centre International de Recherches Dermatologiques Sophia Antipolis, F-06565 Valbonne, Tel. 93 95 70 70, Telex 461030

September 8–10, 1988, Baden (Austria):
22. Wissenschaftliche Tagung der Deutschsprachigen Mykologischen Gesellschaft. Leitung: Prof. Dr. O. Male, I. Univ.-Hautklinik, Alser Str. 4, A-1090 Wien, Tel. 0222/48 00/26 73

September 17–22, 1988, Berlin:
XIVth Congress of the European Academy of Allergology and Clinical Immunology. Ort: International Congress Centrum. Auskunft: Congress Management XIVth Congress of the EAACI, Letzter Hasenpfad 61, D-6000 FrankfurtMain 70, Tel. 069/610474, Telefax 069/610476, Telex 4189353 geco

September 30–October 2, 1988, Lübeck-Travemünde:
62. Tagung der Nordwestdeutschen Dermatologischen Gesellschaft gemeinsam mit der Berliner und der Hamburger Dermatologischen Gesellschaft.
Thema: Infektion an Haut und Schleimhaut.
Leitung: Prof. Dr. Dr. H. H. Wolff.
Aufkunft: Klinik für Dermatologie und Venerologie der Med. Univ. Lübeck, Ratzeburger Allee 160, 2400 Lübeck, Kongreßsekretariat: Tel. 04 51/5 00 25 13 (8–12 Uhr)

October 14–16, 1988, San Francisco/USA:
The 7th Postgraduate Course in Medical Mycology (Dermatology)
Information: Extended Programs in Medical Education, University of California San Francisco, Room U-569, San Francisco, CA 94143-0742.

April 17–20, 1989, Nice/France:
4th European Congress of Clinical Microbiology
Information: I.C.A. International Congress Agency, 4 Villa d'Orléans, F-75014 Paris.