Journal of Antimicrobial Chemotherapy (1985) **16**, 397–399

Short article

Influence of growth phase on the susceptibility of *Candida albicans* to butoconazole, oxiconazole, and sulconazole

William H. Beggs

General Medical Research Service (151), Veterans Administration Medical Center, Minneapolis, Minnesota 55417, U.S.A.

Three topical antifungal imidazoles were examined for fungicidal potential. At 8×10^{-5} M, butoconazole, oxiconazole, and sulconazole were strictly fungistatic against early stationary phase *Candida albicans* cells diluted into fresh medium. With early logarithmic phase organisms, oxiconazole again was fungistatic, but sulconazole and butoconazole were highly lethal at only 2×10^{-5} M.

Introduction

Although imidazole-containing antifungal drugs are primarily fungistatic in effect, there are situations where certain of these agents can exert an impressive fungicidal action. For example, it was recently shown in our laboratory that miconazole at concentrations of 2×10^{-5} M and 4×10^{-5} M (i.e. 8 and 16 mg/l) caused two- to three-log reductions in cfu/ml within 1 h when added to *Candida albicans* cultures in the early stages of exponential growth (Beggs, 1984). When cultures in early stationary phase were diluted and similarly tested, there was no killing effect. Miconazole was strictly fungistatic in this situation. Ketoconazole was only fungistatic regardless of the growth phase of the cells to which it was added. This difference probably reflects the ability of miconazole, but not ketoconazole, to exert direct physicochemical cell membrane damage at moderately high concentrations (Cope, 1980; Sud & Feingold, 1981; Beggs, 1983*a*).

A capacity of antifungal imidazoles for rapid kill of organisms at moderately elevated concentrations would have the greatest probability of clinical relevance in the topical treatment of infections involving the nails, skin, and mucous membranes. The opportunistic fungal pathogen *Can. albicans* is often associated with such infections. Therefore, in this brief study a typical representative strain of *Can. albicans* was used to assess the lethal potentials of three promising imidazole-containing drugs introduced within the past five or six years and developed primarily as topical agents. These drugs include oxiconazole, butoconazole, and sulconazole.

Methods

In our previous related report, the materials and methods used in the present study were described (Beggs, 1984). Included were source and maintenance of *Can. albicans* 11651,

0305–7453/85/090397+03 $02.00/0 © 1985 The British Society for Antimicrobial Chemotherapy

Downloaded from http://jac.oxfordjournals.org/ at University of Iowa Libraries/Serials Acquisitions on May 27, 2015

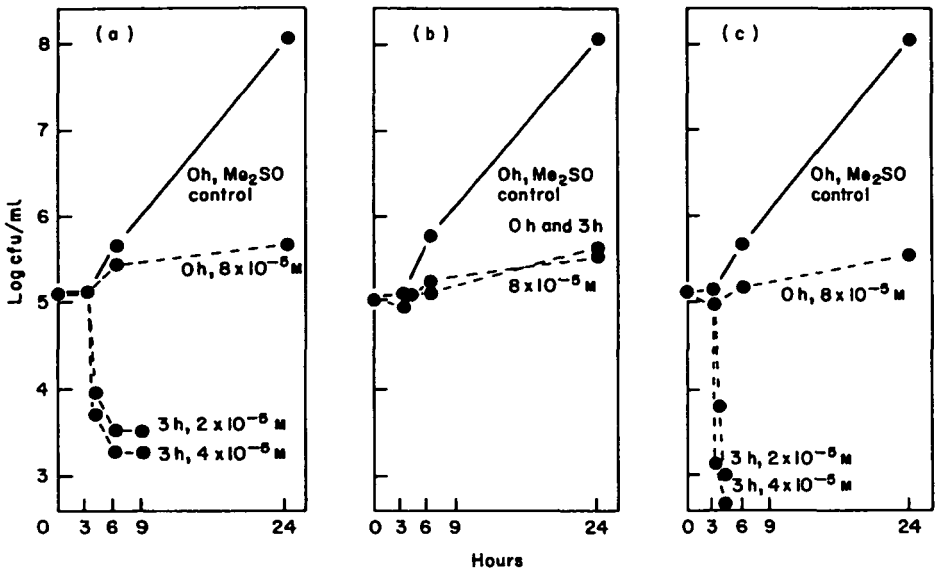

Figure 1. Fungistatic and fungicidal activities of several concentrations of butoconazole nitrate (a), oxiconazole nitrate (b), and sulconazole nitrate (c) when added to *Can. albicans* cultures either at time zero when cells were in stationary phase or at 3 h when cells were in late lag or early logarithmic phase. Me_2SO is drug solvent dimethyl sulphoxide.

composition of our experimental pH 7 synthetic liquid medium, preparation of inocula, cultural conditions, and determination of viability in terms of cfu/ml. *Can. albicans* was grown in 50 ml Erlenmeyer flasks containing 20 ml volumes of medium. Both inoculum and experimental cultures were incubated at 37°C with rotary shaking at 150 r.p.m.

Oxiconazole nitrate was supplied as a gift from Hoffmann–La Roche & Co. Ltd., Basle, Switzerland. Butoconazole nitrate and sulconazole nitrate were gifts from Syntex Inc. Palo Alto, CA. The imidazoles were dissolved in dimethyl sulphoxide at molar concentrations of such strength that at no time did the concentration of solvent added to cultures exceed 1% (v/v). Growth of *Can. albicans* in the absence and presence of solvent at this level was not significantly different.

Results

Results are presented in Figure 1. At a concentration of 8×10^{-5} M, representing approximately 30 mg of imidazole/l, none of the drugs was fungicidal when added together with early stationary phase inoculum cells to fresh medium at time zero. In each case a strong fungistatic effect resulted over 24 h of incubation. Some dramatic differences were noted, however, when cultures were allowed to incubate 3 h prior to the addition of imidazole. At this point *Can. albicans* cells were in the late lag or the very early stages of exponential growth. At concentrations as low as 2×10^{-5} M (i.e. approximately 8 mg of imidazole/l) butoconazole and sulconazole caused precipitous decreases in numbers of cfu/ml. Over a two-log reduction occurred within 1 h with sulconazole (i.e. at 4 h of incubation), and butoconazole caused one- to two-log reductions in viability within 3 h (i.e. at 6 h of incubation). In contrast, oxiconazole failed to exert any measurable lethal activity when added to 3 h cultures at a concentration of 8×10^{-5} M,

Downloaded from http://jac.oxfordjournals.org/ at University of Iowa Libraries/Serials Acquisitions on May 27, 2015

but strong fungistasis was again observed. Whether even higher concentrations of oxiconazole can kill *Can. albicans* in this system was not tested, but it is clear that on a mole for mole basis, the lethal action of sulconazole and butoconazole at moderately high concentrations is far superior to that of oxiconazole.

Discussion

Results presented in this report showed that a capacity to affect strong fungicidal activity against late lag and early logarithmic phase cells of *Can. albicans* at moderately high levels (i.e. 2×10^{-5} M to 4×10^{-5} M) is characteristic of some antifungal imidazoles but not others. Oxiconazole at a concentration of 8×10^{-5} M was only fungistatic and incapable of killing the test organism regardless of growth phase. Its activity was reminiscent of earlier observations in tests with ketoconazole (Beggs, 1984). Oxiconazole and ketoconazole apparently lack a capacity to exert lethal activity, or are possibly able to do so only at high concentrations in excess of 8×10^{-5} M. The growth phase-dependent lethal action of sulconazole demonstrated here was slightly better than that of butoconazole and almost identical to the precipitous and profound decreases in yeast cell viability observed in earlier tests with miconazole (Beggs, 1984). The intensities of fungicidal activity seen with each of the imidazoles studied correlate in a direct manner with earlier data regarding the capacities of these agents for direct physicochemical cell damage (Beggs, 1983*b*). Direct cell membrane damage is the most probable explanation for the growth phase-dependent fungicidal activities of sulconazole and butoconazole as well as miconazole.

Acknowledgement

This work was supported by the Veterans Administration.

References

Beggs, W. H. (1983*a*). Comparison of miconazole- and ketoconazole-induced release of K^+ from *Candida* species. *Journal of Antimicrobial Chemotherapy* **11**, 381–3.

Beggs, W. H. (1983*b*). The effect of antifungal imidazoles on resting cells of *Candida parapsilosis*. *IRCS Medical Science* **11**, 677.

Beggs, W. H. (1984). Growth phase in relation to ketoconazole and miconazole susceptibilities of *Candida albicans*. *Antimicrobial Agents and Chemotherapy* **25**, 316–8.

Cope, J. E. (1980). Mode of action of miconazole on *Candida albicans*: Effect on growth, viability and K^+ release. *Journal of General Microbiology* **119**, 245–51.

Sud, I. J. & Feingold, D. S. (1981). Heterogeneity of action mechanisms among antimycotic imidazoles. *Antimicrobial Agents and Chemotherapy* **20**, 71–4.

(*Manuscript accepted 17 April 1985*)

Downloaded from http://jac.oxfordjournals.org/ at University of Iowa Libraries/Serials Acquisitions on May 27, 2015