Original Article

Comparative Studies on the Postantifungal Effect of
Cispentacin and Pradimicin (BMY-28864)
on *Candida albicans*

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Abstract

The *in vitro* postantifungal effect (PAFE) following exposure of *Candida albicans* to cispentacin and pradimicin (BMY-28864) for short periods of time was investigated. A turbidometric method was used to measure cell regrowth following exposure to different concentrations (0.313 to 20 $\mu\text{g/ml}$) of the two drugs for 0.5, 1 and 2 hr at 30°C and 37°C. The PAFE was determined by the difference in time (hr) required for growth of the control and test cultures to reach the 0.5 absorbance level after removal of the drug by dilution. The length of the PAFE was dependent upon the drug concentration, exposure time and temperature and the strain of *C. albicans* being assayed. Optimal PAFEs ranged from 3.7 to >17.0 hr with cispentacin and from 1.7 to >21.0 hr with pradimicin (BMY-28864).

Key words: postantifungal effect, *Candida albicans*, cispentacin, pradimicin (BMY-28864), antifungal agents, *in vitro* susceptibility assay

Introduction

Many *in vitro* assays have been used to determine susceptibility profiles of fungal organisms to certain drugs, but studies are continuing in an effort to develop improved procedures which will yield results that correlate well with the *in vivo* response to therapy. We have recently been conducting *in vitro* susceptibility studies in our laboratory on the postantifungal effect (PAFE) produced by different antifungal agents on *Candida albi-*

cans^{1,2)}. Numerous studies on this phenomenon have been performed with bacterial organisms (the postantibiotic effect: PAE) during the past several years, but not with fungal organisms³⁻⁶⁾. PAFE determinations provide information concerning the persistent suppression of fungal growth that follows limited exposure (0.5 to 2 hr) of fungal cells to antifungal agents. This is in contrast to most susceptibility assays in which determinations are made after continual exposure of the organism to the drug for 24 hr or longer. PAFE determin-

ations therefore provide susceptibility profile data to the clinician that other assays do not provide, and such information may be useful in selecting an optimal treatment regimen. Viable counts¹⁾ and turbidometric²⁾ methods have been used in our laboratory to evaluate *C. albicans* yeast cell growth following exposure to flucytosine, amphotericin B and miconazole and subsequent removal of the drug by dilution. A PAFE was demonstrated with each agent and generally the duration of the PAFE was dependent upon the agent, the concentration of the agent and the exposure time and temperature^{1, 2)}.

The purpose of the present investigation was to determine if two new antifungal agents, pradimicin (BMY-28864) and cispentacin, could induce PAFEs against *C. albicans* yeast cells. Pradimicin A is the original member of the pradimicins produced by *Actinomadura hibisca*^{7, 8)}. BMY-28864, a water soluble pradimicin derivative, has been shown to have broad and potent antifungal activity against a wide range of pathogenic fungi including *C. albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*⁹⁻¹¹⁾. Cispentacin is a water soluble amphoteric compound produced by *Bacillus cereus* and previous studies have indicated that this simple amino acid-type antibiotic was effective against *C. albicans* and *C. neoformans* in mouse model evaluations^{12, 13)}. In the present study a turbidometric method was used to measure yeast cell growth following limited exposure to different concentrations of the two new antifungals. Comparative evaluations were performed to determine optimal PAFE assay parameters.

Materials and Methods

Antifungal agents: Pradimicin (BMY-28864) and cispentacin were generously provided by Bristol-Myers Squibb Research Institute, Tokyo, Japan. A stock solution of pradimicin (BMY-28864) (2 mg/ml) was prepared in distilled water and methanol added to sterilize the reagent. A stock solution of cispentacin (2 mg/ml) was prepared in methanol. Twofold dilu-

tions of each drug ranging from 20 to 0.313 $\mu\text{g/ml}$ were made in saline for use in the susceptibility determinations.

Organisms: Ten strains of *C. albicans* were used in these investigations. Strain IFM 1001, which is used as a reference strain for susceptibility testing in our laboratory, and clinical isolates 86-23717, 11128-H99, 9319-H76, 9760-H85, 9490-H81, 2773-H26, 3532-K31, 11323-H101 and 1090-2-H17 were maintained on Sabouraud dextrose agar (Eiken Chemical Co., Ltd., Tokyo, Japan) slants. The yeast cells were transferred to yeast nitrogen base broth (Difco Laboratories, Detroit, MI, U.S.A.) containing 1% glucose (YNB-G) and incubated for approximately 24 hr prior to use in the PAFE determinations. Hemocytometer counts were made on the cell suspensions and the yeast cell number was adjusted to approximately 1×10^7 cells/ml for use in the assays.

PAFE assay: Two studies were performed to evaluate the efficacy of pradimicin (BMY-28864) and cispentacin with regard to PAFE production. In the first study optimal exposure time and temperature parameters were determined for each drug using one strain (IFM 1001) of *C. albicans*. The second study was performed to evaluate PAFE production by pradimicin (BMY-28864) (30°C ; 2 hr) and cispentacin (37°C ; 2 hr) against 10 strains of *C. albicans*. Approximately 1×10^7 yeast cells/ml were added (0.2ml) to tubes containing 1.6ml of YNB-G broth and varying concentrations of each drug (0.313 to 20 $\mu\text{g/ml}$) (0.2ml). Controls containing only yeast cells were also used in the assay. The tubes were incubated with shaking at 30°C and 37°C for 0.5, 1 and 2 hr (study one) or at 30°C or 37°C with shaking for 2 hr (study two). Following exposure the drugs were removed by dilution in which the drug-organism suspension was diluted 100 fold in sterile saline. Aliquots (100 $\mu\text{g/ml}$) were added to duplicate wells of a microdilution plate containing 150 μl of YNB-G, placed in a Bioscreen C spectrophotometric instrument (Labsystems, Tokyo, Japan) and incubated for 72 hr at 30°C¹⁴⁾. Growth of the yeast cells

was monitored by the instrument by measuring turbidity (absorbance at 600nm) at 20min intervals. The data were recorded by a computer interfaced with the analyzer and growth curves were generated from each cell preparation. Mean values were determined for each of the cultures from the duplicate growth curve data.

The duration of the PAFE was calculated by a modification of a method used previously for PAE determinations with bacterial systems³⁻⁶⁾ and used in recent PAFE studies in our laboratory^{1,2)}. The formula $PAFE=T-C$ was applied, where T was the time (hr) required for the turbidity of the drug-exposed cell suspension to reach the 0.5 absorbance level after removal of the drug by dilution, and C was the time required for the turbidity of the drug-free control culture to reach the same level. Previous determinations indicated that approximately 1×10^7 viable cells/ml were present when the turbidity of the culture reached the 0.5 absorbance level. Thus $T-C$ denotes the time interval during which an antifungal agent is able to produce a PAFE on fungal growth.

Results

Initial comparative evaluations were performed with the two antifungals to determine optimal PAFE assay parameters. *C. albicans* yeast cells (strain IFM 1001) were exposed to both drugs at concentrations ranging from 0.313 to 20 µg/ml for 0.5, 1 and 2 hr at 30 °C and 37°C. In most instances the duration of the PAFE was prolonged as the concentration of each agent and the drug-organism exposure time was increased. The results indicated that optimal PAFEs were achieved when the yeast cells were exposed to cispentacin and pradimicin (BMY-28864) for 2 hr at 37°C and 30°C, respectively (Table 1 and 2). Cispentacin produced PAFEs ranging from 0.8 to 11.8 hr at 30°C and from 2.4 to 16.5 hr at 37°C (Table 1). In contrast, PAFEs with pradimicin (BMY-28864) ranged from 0.2 to 11.0 hr at 30°C and from 0 to 8.8 hr at 37°C (Table 2). PAFEs of shorter duration were induced when the cells

Table 1. PAFE (hr) following exposure of *C. albicans* strain IFM 1001 to various concentrations of cispentacin for different time intervals at 30°C and 37°C

Concentration (µg/ml)	Exposure time (hr)					
	0.5		1		2	
	30°C	37°C	30°C	37°C	30°C	37°C
0.313	0	0.2	0.7	0.8	0.8	2.4
0.625	0	0.2	0.8	1.5	1.3	3.6
1.25	0.3	0.5	1.2	2.5	1.8	4.7
2.5	0.5	1.3	1.7	4.0	3.3	7.1
5	0.7	3.0	3.3	6.5	4.1	9.8
10	2.2	5.2	4.9	11.0	8.4	12.7
20	3.3	9.1	8.3	15.2	11.8	16.5

Table 2. PAFE (hr) following exposure of *C. albicans* strain IFM 1001 to various concentrations of pradimicin (BMY-28864) for different time intervals at 30°C and 37°C

Concentration (µg/ml)	Exposure time (hr)					
	0.5		1		2	
	30°C	37°C	30°C	37°C	30°C	37°C
0.313	0.2	0	0	0	0.2	0
0.625	0.3	0.2	0.3	0	0.7	0.3
1.25	0.5	0.3	0.3	0.3	0.8	0.3
2.5	0.8	0.7	0.8	0.3	1.2	0.8
5	1.2	0.8	1.3	1.0	2.3	1.5
10	2.4	2.3	4.4	2.6	5.6	4.1
20	4.1	4.0	6.0	6.3	11.0	8.8

were exposed to the two drugs for periods of 0.5 and 1 hr.

In the second study the efficacy of the two agents with regard to PAFE production against 10 strains of *C. albicans* was evaluated (Tables 3 and 4). PAFE values obtained with the four concentrations of cispentacin ranged from 0.2 to 3.7 hr (0.313 µg/ml), 0.7 to 9.0 hr (1.25 µg/ml), 3.3 to 15.3 hr (5 µg/ml) and 5.7 to >17.0 hr (20 µg/ml) (Table 3). Pradimicin (BMY-28864) induced PAFEs ranging from 0.2 to 1.7hr (0.313 µg/ml), 0.3 to 3.3 hr (1.25 µg/ml), 2.3 to 10.0 hr (5 µg/ml) and 10.0 to >21.0 hr (20 µg/ml) (Table 4). Thus both agents produced suppressive effects on yeast

Table 3. PAFE (hr) following exposure of *C. albicans* (10 strains) to four concentrations of cispentacin for 2 hr at 37°C

Strain	Cispentacin concentration ($\mu\text{g/ml}$)			
	0.313	1.25	5	20
1001	1.7	4.0	9.0	16.7
86-23717	1.0	2.3	3.7	10.0
11128-H99	1.0	1.8	3.7	8.3
9319-H76	0.2	0.7	3.0	5.7
9760-H85	0.7	2.5	4.0	7.0
9490-H81	0.3	1.0	3.7	8.3
2773-H26	0.7	1.7	3.3	6.0
3532-K31	1.0	2.0	5.3	12.3
11323-H101	0.3	2.0	4.0	7.7
1090-2-H17	3.7	9.0	15.3	>17.0

Cell growth turbidity did not reach the 0.5 absorbance level during the evaluation period when strain 1090-2-H17 was exposed to 20 $\mu\text{g/ml}$ of the drug.

Table 4. PAFE (hr) following exposure of *C. albicans* (10 strains) to four concentrations of pradimicin (BMY-28864) for 2 hr at 30°C

Strain	Pradimicin concentration ($\mu\text{g/ml}$)			
	0.313	1.25	5	20
1001	0.3	0.7	2.3	10.0
86-23717	0.3	0.7	3.3	10.3
11128-H99	0.2	0.3	6.7	14.3
9319-H76	0.3	1.0	3.0	11.7
9760-H85	1.7	2.3	10.0	>21.0
9490-H81	0.3	1.3	5.3	15.7
2773-H26	0.2	0.5	4.7	15.3
3532-K31	0.2	0.3	7.0	11.7
11323-H101	0.3	2.0	8.3	18.7
1090-2-H17	1.7	3.3	7.7	16.7

Cell growth turbidity did not reach the 0.5 absorbance level during the evaluation period when strain 9760-H85 was exposed to 20 $\mu\text{g/ml}$ of the drug.

cell growth and the duration of the PAFE varied according to the strain of *C. albicans* being assayed.

Discussion

Many new antifungal agents have been deve-

loped in recent years in attempts to meet the ever increasing demand for improved therapeutic measures. In conjunction with this many new *in vitro* and *in vivo* susceptibility investigations have been performed in an effort to develop improved laboratory methods for determining susceptibility profiles of fungal organisms to certain drugs and to ultimately aid clinicians in decisions on optimal therapeutic regimens¹⁵⁾. Many laboratories have been performing MIC determinations as well as other susceptibility assays, but the results from the assays can vary appreciably depending upon such factors as the strain of the organism, exposure time, incubation temperature, media composition, etc¹⁵⁻¹⁷⁾. In most standard *in vitro* susceptibility assays determinations are made following continual exposure of the organism to a particular drug for a period of 24 hr or longer, but in the *in vivo* situation organisms are exposed to fluctuating and not constant drug levels. Generally the goal with regard to treatment has been to attempt to maintain the serum drug concentration above the MIC for the entire dosing interval, but only a few studies have been performed to determine if such levels are required for optimal dosing efficiency. This critical question needs to be resolved in the future and information gained will be of paramount importance to clinicians regarding treatment schedules.

A number of *in vitro* susceptibility studies with bacteria have indicated that certain antibiotics exert profound suppressive effects on cell growth after only a short period of exposure of the antimicrobial to the organism. This period of persistent suppression of bacterial growth following removal of the agent has been termed the postantibiotic effect as mentioned above. This type of assay, in which organisms are not continually exposed to the antibiotic, may better approximate the actual *in vivo* situation than some of the standard MIC assays.

We have recently begun investigations to determine if suppression of fungal growth results from limited exposure of an organism to a

particular antifungal agent. As stated earlier we have been successful in demonstrating a postantifungal effect or PAFE, as we have termed this activity, with flucytosine, amphotericin B and miconazole against *C. albicans*^{1,2)}. In addition, preliminary investigations with combinations of flucytosine and fluconazole have indicated that the synergistic interaction of the two drugs, at concentrations considerably lower than their individual MICs, produced PAFEs of longer duration than those produced by either agent alone (unpublished observations).

In the present study with the two new antifungal agents cispentacin and pradimicin (BMY-28864) we have also been able to demonstrate a suppressive effect produced by different concentrations of the agents on 10 strains of *C. albicans*. Optimal PAFEs from 3.7 to >17.0 hr were obtained when four concentrations of cispentacin, ranging from 0.313 to 20 µg/ml, were assayed (2 hr exposure time: 37°C). Similar suppressive effects were also experienced when the 10 strains were exposed to the same four concentrations of pradimicin (BMY-28864) for 2 hr at 30°C. Optimal PAFEs ranging from 1.7 to >21.0 hr were produced.

The results from this and our previous studies are encouraging with regard to further investigations and development of this *in vitro* susceptibility method. Such an assay may provide clinicians with additional susceptibility information that may be useful in determining optimal dosing regimens. Theoretically the presence or absence of a PAFE should provide a rationale for either less frequent drug administration, where the drug concentration decreases below the MIC, or more frequent administration of the particular antifungal agent. It is of importance to keep in mind that PAFE determinations are performed *in vitro* and that *in vivo* investigations will need to be performed to further define the applicability of this procedure as a method of determining dosing regimens of antifungal drugs.

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