Interaction among Nystatin and Penicillin G against *Saccharomyces Cerevisiae*

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Summary
MICs of nystatin and penicillin G were determined for *Saccharomyces cerevisiae*, alone and in combination. By using growth control for comparison, broth macrodilution testing was performed according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Nystatin x penicillin G was a synergistic fungicidal combination against the yeast. The synergy was effective when 100 IU penicillin G /ml was used with both 25 and 100 IU nystatin /ml. However, at lower concentration, nystatin was unable to exert its fungicidal action on growth of *S.cerevisiae* on the duration of exponential phase.

Key words: nystatin, penicillin G, synergism, *Saccharomyces cerevisiae*

Introduction

When tissue culture may be contaminated by fungi (yeast, molds) or bacteria, it is advisable to supplement tissue culture media with antibiotics. The mechanisms of action of most antibacterial drugs is not completely understood, It is convenient to present these mechanisms of action under four headings: 1) alternation of cell membrane permeability, 2) inhibition of cell wall synthesis, 3) inhibition of protein synthesis, and 4) inhibition of nucleic acid synthesis.

One possible reason for employing 2 or more antimicrobials simultaneously instead of single drug is to achieve microbial synergism. One drug may affect the cell membrane and facilitate the entry of the second drug, one drug may enhance the uptake of a second drug, or the other types of situations. Often the synergistic effect permits significant reduction in dose and thus avoids toxicity (Jawetz 1992). Synergy between non-antifungal and antifungal agents is not without precedent (Shahan & Pore 1991, Warnock *et al* 1989a).

In the present study, the effect of penicillin G and nystatin on *Saccharomyces cerevisiae* alone and in combination was compared.

Materials and Methods

Organism. *Saccharomyces cerevisiae* was obtained from our diagnostic laboratory in Foot-and-Mouth Disease (FMD) department, isolated from an infected BHK-21 cell culture.

Antimicrobial agents. Nystatin was purchased from Squibb & Sons, New York, USA and was kept as a 1mg/ml stock in N,N-dimethylform-amid (Merck) at -25C. Penicillin G was purchased from Jaber Ebn Hayyan, Tehran, Iran. Stock solution (1mg/ml)was prepared in distilled water.

Medium. The Earl medium with BHK-21 cells, pH 6.6±1, was used.

Growth curves. Four flasks with equal volumes of Earl medium with BHK-21 cells and various concentration of antibiotics were chosen. Flasks 1-3 contained 100IU nystatin, 100IU penicillin G, and 100IU nystatin x100IU penicillin G/ml respectively; flask 4 was, without any drug, as control. All of them were inoculated by $1x10^6$ cells/ml of *S.cerevisiae,* the yeast inoculum was prepared from overnight culture on Sabouraud's dextrose agar (Difco), and incubated at 36±1C on water bath and shacked for 20h. Four samples were taken from cultures periodically and diluted 10^{-3} with warm and fresh Earl medium. The drugs were removed by washing. Viable counts for each sample were performed by plating serial ten-fold dilutions on Sabouraud's dextrose agar. Control culture was subjected to the same procedures in each experiments. Number of viable cells per ml were determined and plotted, four curves were obtained. Each count was the average of 3 replicated plates.

Macrodilution susceptibility test. Broth macrodilution testing was performed according to CCLS guidelines (Warnock 1989b). Dilutions were prepared in 1ml of Earl; the medium was $1x10^6$ *S.cerevisiae* cells. The final concentration ranges of the antibiotics were 25-200 IU/ml for nystatin and 50-300IU/ml for penicillin G. The tubes were incubated at 36±1 C for 24h. The reading criterion was the lowest concentration at which there was no fungal growth after incubation. Growth must not presented in the growth control (drug free) tubes.

Checkerboard assay. The checkerboard method for antimicrobial combination, two at a time, was used (Berehbaum 1978). Seven flasks with equal volumes of Earl medium and concentrations of BHK-21 cells were chosen. In different dilutions, in combination and singly, of nystatin and penicillin G number of dead cells per ml were determined and killing cells percentages were calculated. Each experiment was done four times and standard deviation of the means (SD) was also applied to this assay.

Results

The influence of two antimicrobial agents on growth of *S.cerevisiae* is shown in Figure 1. The growth constant, *K*, for each experience was calculated. As expected, penicillin G had a weak effect alone, but appeared to have a synergistic effect with nystatin when both concentrations were 100IU/ml.

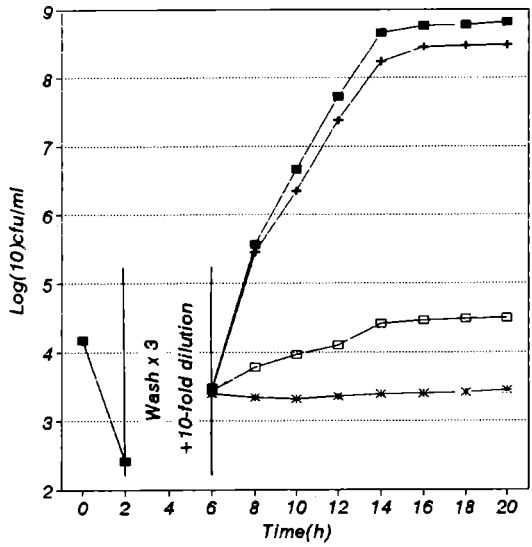

Figure 1. *Antifungal activity of 100IU/ml nystatin and penicillin G on S.cerevisiae, in the presence of BHK-21 cells.* ■,*control;* +,*penicillin G,*□,*nystatin;* *,*penicillin x nystatin*

The MICs as determined by broth macrodilution method of two drugs tested against *S.cerevisae* are 50, 300, 25x100(IU/ml) for nystatin, penicillin G and nystatin x penicillin G, respectively.

Nystatin was active (24h-MIC for the isolate tested=50IU/ml) penicillin G was least active (24h-MIC for the isolate tested=300IU/ml) and in combination, were must active (24h-MIC for the isolate tested=25 and 100IU/ml, respectively). The fractional inhibitory concentration (FIC) was calculated by the formula:

FIC=MIC of drug A in combination/MIC of drug A alone+
MIC of drug B in combination/MIC of drug B alone

If the sum of these fractions is less than 1, the combination is synergistic and if it is greater than 1, the combination is antagonistic.

According to the results of MIC the growth curves were obtained with both agents (Fig.2).

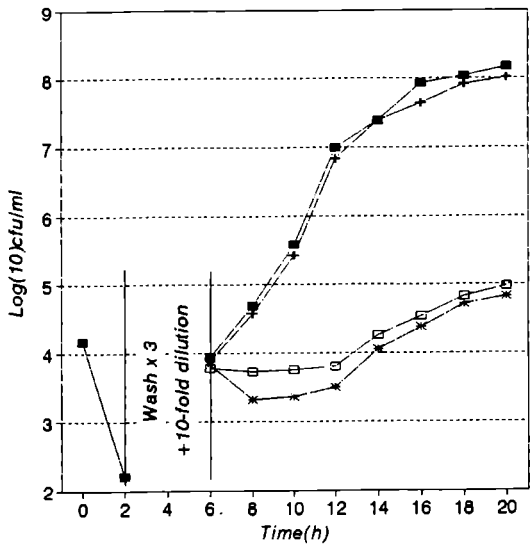

Figure 2. *Antifungal activity of 25IU/ml nystatin and 100IU/ml penicillin G on S.cerevisiae, in the presence of BHK-21 cells.* ■,*control;* + ,*penicillin G;*□,*nystatin;* • ,*penicillin x nystatin*

By checkerboard assay each drug was tested alone on range of MIC which was observed, and the effect of the combination was compared to the individual results to seen. Penicillin G have had synergistic activity with nystatin at concentrations of 100 and 25IU/ml respectively, the four-fold increase in the mean percentage killed (60.15%) had a light SD of 1.42 (Fig.3).

Discussion

Nystatin for use as a fungicide in cell culture, is a polyene antibiotic. According to the manufacturer's instructions (Squibb Ins.) activity dose decrease when aqueous suspensions are stored at 37C in various tissue culture media. For example the activity of this drug in Ziegler's modification of Earl medium [with 10 % (v/v) calf

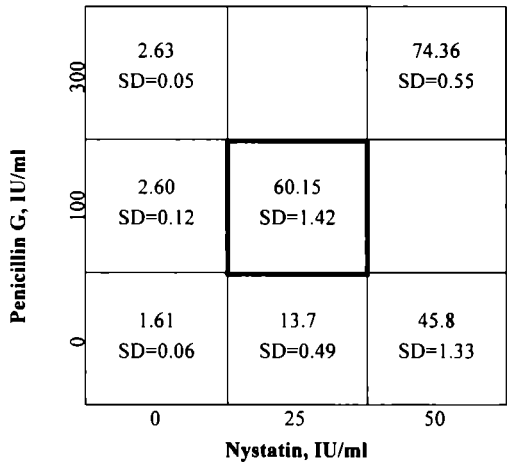

Figure 3. *Chckerboard assay of S. cereviciae; percentage of yeast killed in squares. Nystatin x penicillin G, synergistic drug concentrations effect designated by heavy line.*

serum] decrease from 53 to 25 on 1 day, and to 8 on 2 days at 37C. So, additional nystatin must be added to cell culture media in long-term experiments to maintain a level of antifungal activity. Nystatin does not significantly effect virus propagation but additional dose may inhibit the growth and propagation of some viruses. Among 15 strains of Foot-and-Mouth-Disease viruses, the growth of 1 strain in epithelial tissue of cattle tongue is inhibited by 80IU/ml nystatin (Squibb Ins.). Thus it is necessary to avoidance of contamination treat cell culture media with a combination of antimicrobial agents. 100 units penicillin G per ml is recommended for supplement tissue culture media. This is a bacterial cell wall inhibitor and known that not bound by yeast, but little absorption (about 10-20%) of penicillin may be observed when the yeast cells were incubated with complex media, pH 6.1-6.8, containing carbohydrate and salts. MIC of penicillin G was found to be least in Earl medium. As to be shown in figures 1 and 2, a combination of nystatin and penicillin G was active, *K*, the growth rate constant, were 0.04^{-1}h and 0.02^{-1}h respectively.

The FIC calculated such as:

$$FIC=100/300+25/50=0.83$$

Because of the FIC is less than 1 the combination, 25IU/ml nystatin and 100IU/ml penicillin G, is synergistic.

The checkerboard technique was used to confirm this synergy effect. Its data are presented as of yeasts killed. Figure 3 shows percentage of yeast killed in squares. Nystatin alone, killed 13.7% of the yeast at 25 units concentration per ml and killed 60.15% of the yeast at the same concentration when combined by 100IU/ml penicillin G. The fourfold increase is the mean percentage killed cells had a light SD of 1.42. It is observed that when nystatin was used together with penicillin G, synergism could observed. Better results were obtained for *S.cerevisiae*, which was clearly killed by synergistic rations of the two antibiotics.

Comparison of two growth curves shows exposure of the *S.cerevisiae* to 25IU/ml nystatin causes regrowth relative to the other exposed yeast culture (100IU/ml). Nystatin binds to sterols on the fungal cell membranes and disturbs their function (Pore 1990). The length of time required for the membrane to be damaged is approximately 5h, and a 9h incubation period led to optimal separation of damaged. Antifungal susceptibility is influenced by both the medium itself and its pH, and undamaged cells. The culture continue to grow slowly after 12h, may be in order to nystatin is unstable when incubated at 37C and rather rapid inactivated by acid which is produced in Earl medium by growth of both yeast and BHK-21 cells. Thus, in such conditions, to maintain a level of antifungal activity, the concentration of nystatin should be adjusted.

Acknowledgment

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