Differential Potentiation by Nystatin of the Effect of Antibiotics on Yeast and Mammalian Cells

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The effect of the polyene antibiotic nystatin, used in combination with several other antibiotics, on the membranes of Candida albicans SC#8169 (yeast) cells and of Chinese hamster ovary and 3T3 (mammalian) cells was investigated. It was concluded from determinations of the viability of the yeast cells and from measurements of deoxyribonucleic acid synthesis by the mammalian cells that nystatin acts synergistically with several antibiotics on yeast cells, but not on CHO and 3T3 cells. This selective action of nystatin may prove useful in chemotherapy.

Several reports (5, 6, 9) have dealt with potentiation by the heptaene antibiotic amphotericin B of the effect of various chemotherapeutic agents on yeast cells. The mechanism of this potentiation is thought to involve formation of a hole in the yeast cell membrane (10), thus facilitating entry of the chemotherapeutic agent. This hole is apparently formed because amphotericin B combines with membrane sterols, and the physical state of the membrane is altered (8). We wish to report that nystatin, the tetaene antibiotic, also potentiates the effect of several chemotherapeutic agents on the yeast Candida albicans SC#8169 (C. albicans), but not on Chinese hamster ovary (CHO) and 3T3 cells. Nystatin potentiated the entry into C. albicans cells of 5-F cytosine, tetracycline, rubiflavin (1), toycamycin, and actinomycin D, but not of polymixin B, streptothricin, or gelbeidine (A. Aszalos, R. Robinson, S. Giannini, B. Berk, and J. Alicino, Prog. Abstr. Internci. Conf. Antimicrob. Agents Chemother., 8th, New York, p. 16, 1968). These conclusions are based on studies of cell viability and on measurements of leakage of ultraviolet (UV) (260 nm)-absorbing materials from the treated cells. The effect of actinomycin D, toycamycin, rubiflavin, or tetracycline on CHO and 3T3 cells, as measured by incorporation of radioactive precursors into deoxyribonucleic acid, (DNA), ribonucleic acid (RNA), and proteins, was not potentiated by nystatin.

MATERIALS AND METHODS

Measurement of leakage of UV-absorbing material from C. albicans cells. For the measurement of leakage of UV (260 nm)-absorbing materials from drug-treated C. albicans, the yeast cells from a flourishing slant growth were propagated in a medium containing, per liter, 10 g of glucose, 3 g of yeast extract, and 10 g of KCl. After 24 h of shake-flask incubation, the cells were centrifuged at 10,000 rpm for 15 min. The supernatant was decanted and the cells were resuspended in sterile phosphate buffer, pH 6.8, then centrifuged again. After decantation of the supernatant, cells were again suspended in phosphate buffer to a dilution having an optical density of 4.5 at 660 nm. The yeast suspension was divided into 100-ml aliquots and the test compounds, each dissolved in methanol, were added. Aliquots of 5 ml were taken at different times and filtered on membrane filters (Millipore Corp.). The optical density of the filtrate was determined at 260 nm with a Perkin Elmer 139, UV-VS spectrophotometer. Methanol, up to 5% (vol/vol), had no effect on the leakage of UV (260 nm)-absorbing materials, as determined in separate experiments.

Viability of C. albicans cells. Viability studies were carried out in the same way as the leakage studies, up to the point of addition to the yeast cell suspension of the drugs in methanolic solution. After this addition, aliquots were taken at different times and diluted serially with phosphate buffer, pH 6.8. From each dilution, a 1-ml aliquot was added to a 10-cm diameter petri dish that contained 10 ml of the medium used for the propagation of the yeast cells (see Fig. 1), plus agar. Petri dishes were incubated for 24 h at 37 C and the yeast colonies were counted. The dilution that yielded no more than two colonies per petri dish was designated the end point in the dilution series; end-point dilutions were plotted against time.

The incorporation of radiolabeled precursors into the DNA, RNA, and proteins of the mammalian cells was determined according to the method of Kuwano et al. (7).

In the culturing and handling of CHO cells, the technique described by Sanders and Pardee (13) was followed, except that Linbro dishes were used instead of glass cover slips. In the handling of 3T3 cells, the methodology of Foster and Pardee (2) was used, but Linbro dishes were again used.
RESULTS

Effects of nystatin on C. albicans cells.
Figure 1 shows the effect of different concentrations of nystatin on the leakage of UV (260 nm)-absorbing materials from C. albicans. Figures 2 and 3 show the effects of the combined actions of nystatin and actinomycin D or tetracycline, respectively. These results indicate that actinomycin D or tetracycline acting with nystatin causes greater leakage of UV (260 nm)-absorbing materials from C. albicans cells than does nystatin alone. The concentrations of actinomycin D and tetracycline used are themselves without effect.

Comparable results were obtained when the viability of yeast cells was measured after their exposure to nystatin alone and to nystatin in combination with actinomycin D or tetracycline (Fig. 4 and 5).

Like actinomycin D and tetracycline, 5-F cytosine, rubiflavin, and toyocamycin also act synergistically with nystatin, resulting in viability curves for C. albicans similar to those shown in Fig. 4 and 5. In these studies, the antibiotics were tested at concentrations of 15, 20, and 30 \( \mu g/ml \), respectively, combined with nystatin, 15 \( \mu g/ml \). However, streptomycin, gelbeidine, and polymyxin B, at concentrations of 10, 25, and 25 \( \mu g/ml \), respectively, showed no evidence of synergism with nystatin, 15 \( \mu g/ml \).

Effects of nystatin on mammalian cells.
The possible potentiation by nystatin of the effect of actinomycin D, tetracycline, rubiflavin, gelbeidine, or toyocamycin on CHO and 3T3 cells was studied in cell culture systems. First, we determined the maximal concentration of nystatin that did not affect the rate of incorporation of radiolabeled thymidine, uridine, and leucine into DNA, RNA, and proteins, respectively. It was found that, at concentrations of 20 and 10 \( \mu g/ml \), nystatin did not affect the rate of biosynthesis of these polymers in CHO and 3T3 cells, respectively. These concentrations of nystatin correspond to the 15 \( \mu g/ml \) used in studies of the viability of yeast cells.

We determined the concentration of each of the six antibiotics that either did not inhibit incorporation of radiolabeled precursors into DNA, RNA, or proteins of CHO and 3T3 cells, or caused, at most, a 10 to 15% inhibition. These concentrations are given in Table 1. When these concentrations of the antibiotics were combined with nystatin, 20 and 10 \( \mu g/ml \) for CHO and 3T3 cells, respectively, the incorporation of radiolabeled precursors into CHO or 3T3 cells was not altered.

DISCUSSION

The results of the experiments reported here indicate that the action of nystatin on the yeast cell membrane makes it possible for the antibiotics 5-F cytosine, tetracycline, rubiflavin, toyocamycin, and actinomycin D to exert their effects, each according to its known mode of action (3). Since these antibiotics differ in their
of the effect of the antibiotic on yeast cells.

Although nystatin potentiates the effects of several drugs on yeast cells, as does amphotericin B, it does not potentiate the effects of these same drugs on CHO and 3T3 mammalian cells.

A selectivity for potentiation of the effects of drugs on yeast cells is not shown by amphotericin B, which potentiates the effect of several drugs on yeast (5, 6), and the effect of chromomycin A3 (similar to gelbecidine) and bleomycin on transformed fibroblastic cells (7), and of rifamycin and tetracycline on mouse L cells and human HeLa cells (11) and on 3T3 cells (12).

Table 1. Concentrations of drugs that did not affect or affected only slightly the synthesis of DNA, RNA, and protein in CHO and 3T3 cells

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>CHO cells</th>
<th>3T3 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycin D</td>
<td>0.5</td>
<td>30</td>
</tr>
<tr>
<td>Gelbecidine</td>
<td>1000</td>
<td>5000</td>
</tr>
<tr>
<td>Rubiflavin</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Streptothricin</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>Toyocamycin</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>

*As determined by Kuwano et al. (7). The drugs were dissolved in Me2SO and diluted with the culture medium, so that <0.5% Me2SO was present in the final medium.
We conclude from our experiments with nystatin and from those of others with amphotericin B that the potentiation of the effect of an antibiotic by another substance will be affected by the potentiator, the specific antibiotic, and the particular membrane system. The selective potentiation by nystatin of the effect of antibiotics on yeast cells, but not on mammalian cells, may find useful application in chemotherapy. Hauchen and Feingold (4) reached a similar conclusion from their experiments with nystatin, amphotericin B, and filipin, a pentane antibiotic, on the action of these polyenes on C. albicans cells and on the membranes of human erythrocytes.

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LITERATURE CITED