Inhibitory Effects of Chlorpromazine on Candida Species

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Chlorpromazine was tested for antifungal activity by using Candida albicans and standard assays. The MIC of chlorpromazine was 35 µg/ml; the minimal fungicidal concentration was also 35 µg/ml. The minimal effective concentration was 2.2 to 3.5 µg/ml (using assays based on quantitative cultures and growth). There was a slight positive interaction between chlorpromazine and amphotericin B but no interaction between chlorpromazine and rifampin. Chlorpromazine also inhibited C. krusei, C. parapsilosis, C. tropicalis, and Torulopsis glabrata. We conclude that phenothiazines have direct anti-Candida activity and that these drugs appear to have a broad antimicrobial spectrum.

Phenothiazines are widely used antipsychotic drugs which have multiple cellular effects, including modification of membranes, alteration of cyclic nucleotide metabolism, and intercalation into DNA (7, 8, 15). Some of these effects may develop when the drugs bind to and inhibit calmodulin, a ubiquitous protein which regulates many intracellular processes (14). Consequently, phenothiazines have therapeutic applications and side effects which do not necessarily involve the central nervous system. For example, these drugs have direct antibacterial activity (9). In addition, Pearson et al. (11) recently demonstrated that chlorpromazine (CPZ) can inhibit the growth of Leishmania donovani in human macrophages, and Schuster and Mandel (12) reported that phenothiazines have in vitro activity against the pathogenic free-living amoeba Naegleria fowleri. Since Candida albicans has a calmodulin-like protein (6), we decided to test CPZ for antifungal activity.

MATERIALS AND METHODS

Candida species. Clinical isolates of Candida albicans, C. krusei, C. parapsilosis, C. tropicalis, and Torulopsis glabrata were obtained from the clinical microbiology laboratory at the University of Iowa Hospitals and Clinics. The cultures were maintained in brain heart infusion agar (Difco Laboratories, Detroit, Mich.) and were grown in tryptic soy broth (TSB) (Difco) at 37°C for 20 h in stationary culture as needed. Overnight cultures were collected by centrifugation (150 × g for 10 min), washed twice with sterile phosphate-buffered saline (pH 7.4), and resuspended in phosphate-buffered saline for use in experiments described below.

Candida growth. Washed C. albicans from an overnight culture was diluted into TBS to a concentration of 10⁵ blastoconidia per ml and incubated at 37°C in the presence of various concentrations of CPZ. Growth was measured by recording the optical density at 550 nm with a Bausch & Lomb Spectronic 20 apparatus.

Germ tube formation. To study the effect of CPZ on germ tube formation, we diluted washed Candida suspensions into newborn calf serum (GIBCO Laboratories, Grand Island, N.Y.) and incubated these suspensions at 37°C with and without CPZ. At various times, samples were withdrawn and fixed with an equal volume of 1% glutaraldehyde in phosphate-buffered saline. The fraction of yeast forms developing germ tubes was determined by phase microscopy (×200 magnification), using morphological criteria described by Soll et al. (13).

MIC-MFC determinations. The MIC and the minimum fungicidal concentration (MFC) of CPZ for each of the fungal species listed above were determined. Serial twofold dilutions of CPZ in TSB (initial concentration, 75 µg/ml) were inoculated with C. albicans to produce a final concentration of either 10³ or 10⁴ blastoconidia per ml. The other Candida species were tested with only 10² blastoconidia per ml as the starting inoculum.Suspensions were then incubated at 37°C for 24 h, and the lowest concentration of CPZ which prevented growth was defined as the MIC. The MFC, defined as the lowest concentration producing a 100-fold reduction in viable CFU, was determined by plating serial dilutions from tubes with no visible growth.

Interaction with amphotericin B. To determine whether CPZ interacts with amphotericin B, the MIC was determined for combinations of the drugs by using a standard checkerboard titration. Suspensions (initial inoculum, 10⁵ blastoconidia per ml) were incubated at 37°C for 24 h, and the MIC was determined for each drug alone and for the various combinations. Amphotericin B was tested in a range from 0.025 to 0.4 µg/ml. Similar experiments were performed with CPZ and rifampin. Rifampin was tested in a range from 1.25 to 10 µg/ml. The criteria of Berenbaum (1) were used to define synergism.

Drugs. Stock solutions of CPZ (5 mM) were prepared fresh daily with crystalline CPZ hydrochloride (Sigma Chemical Co. St. Louis, Mo.) and double-distilled water. Stock solutions of amphotericin B (1 mg/ml) were prepared by dissolving amphotericin B (Sigma) in dimethyl sulfoxide and stored at 0°C. Rifampin (Sigma) was dissolved in ethanol and

<table>
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<tr>
<th>TABLE 1. MIC and MFC for Candida species</th>
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<tr>
<td><strong>Species (inoculum density/ml)</strong></td>
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<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td>C. albicans</td>
</tr>
<tr>
<td>10⁻</td>
</tr>
<tr>
<td>10⁰</td>
</tr>
<tr>
<td>C. krusei</td>
</tr>
<tr>
<td>C. parapsilosis</td>
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<tr>
<td>C. tropicalis</td>
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<tr>
<td>T. glabrata</td>
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* Results represent data from three to seven experiments for each isolate.

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stored at 4°C. All solutions were protected from light and diluted into phosphate-buffered saline before a final dilution into TSB.

RESULTS

MIC and MFC. We initially determined the MIC and MFC of CPZ for a clinical isolate of C. albicans. These studies demonstrated that the MIC depended slightly on the initial inoculum density and that the MFC was similar to the MIC (Table 1). When these experiments were repeated with yeast nitrogen base medium (Difco), the MIC was 35 to 70 μg/ml.

Minimal effective concentration. To determine whether chlorpromazine had anti-Candida effects at lower concentrations, we examined the effect of chlorpromazine on Candida growth by turbidometric and plate count assays. CPZ inhibited Candida growth in TSB in a dose-dependent manner (Fig. 1): 3.5-μg/ml concentrations consistently and significantly reduced growth slightly (P < 0.05 for paired t test of data at 4, 6, and 8 h of growth). This inhibition did not depend on the growth phase, and 35 μg of chlorpromazine per ml inhibited cultures in the exponential phase of growth (data not shown). In addition, inhibition did not depend on light exposure (data not shown), and 2 mM Ca²⁺ partially reversed the inhibition (Fig. 1). CPZ effects were also measured with quantitative cultures. In this assay, lower concentrations (2.2 μg/ml) significantly reduced growth in TSB (Table 2).

Other effects. CPZ (35 μg/ml) reduced germ tube formation in newborn calf serum (Table 3) but did not inhibit chlamydomere formation.

Other Candida species. CPZ also inhibited the growth of C. krusei, C. parapsilosis, C. tropicalis, and T. glabrata (Table 1). It did not kill C. parapsilosis.

Interaction between CPZ and amphotericin B. We used a checkerboard technique to test for an interaction between chlorpromazine and amphotericin B, using the same C. albicans isolate. This isolate had an MIC of chlorpromazine of 35 μg/ml and of amphotericin B of 0.4 μg/ml. There was a slight but definitely positive interaction between these two drugs (the sum of the fractional inhibitory concentrations was 0.75, n = 3 determinations) (1). Rifampin (10 μg/ml) had no effect on Candida growth in the presence of chlorpromazine (35 μg/ml).

DISCUSSION

These studies demonstrated that CPZ has fungistatic and fungicidal activity against C. albicans and that the minimal effective concentration approaches 2 μg/ml. CPZ also inhibited germ tube formation, an active synthetic process which requires DNA, RNA, and protein synthesis, and hence had effects on morphological differentiation in addition to effects on growth. The mechanism for these antifungal effects is unknown. CPZ may have direct effects on C. albicans membranes, similar to the postulated mechanism for the antibacterial properties, or it may inhibit some aspect of Candida metabolism by binding to calmodulin (6, 9). CPZ had a synergistic interaction with amphotericin B but not with rifampin.

These CPZ effects on Candida growth and differentiation may have use in studies on fungal physiology and pharma-

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TABLE 2. Candida growth in TSB

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<thead>
<tr>
<th>CPZ (μg/ml)</th>
<th>Mean CFU ± SE (fraction of control)</th>
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<tr>
<td>2.2</td>
<td>0.57 ± 0.09</td>
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<tr>
<td>4.4</td>
<td>0.68 ± 0.10</td>
</tr>
<tr>
<td>8.8</td>
<td>0.49 ± 0.06</td>
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<tr>
<td>17.5</td>
<td>0.25 ± 0.04</td>
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<tr>
<td>35.0</td>
<td>0.01</td>
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* The number of CFU after 24 h of growth in TSB in presence of CPZ is expressed as the fraction of the number of CFU in control cultures. The initial inoculum was 10⁶ blastoconidia per ml. Results are from four to six separate experiments.

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TABLE 3. Germ tube formation

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<tr>
<th>Cultures</th>
<th>% Germ tube formation after incubation time (min) of:</th>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>1.6 ± 0.9</td>
</tr>
<tr>
<td>CPZ**</td>
<td>—c</td>
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* Percent of blastospores with germ tubes (mean ± standard error).
** 35 μg/ml.
*— Not done.
* Significantly different than control by t test, P < 0.05.
ology. Phenothiazines may also have therapeutic applications in fungal diseases. The concentrations used in these experiments are higher than the concentrations usually achieved in patients (0.1 to 0.5 μg/mL), but phenothiazines may be concentrated as much as 70-fold in tissues such as brain (4). However, any therapeutic application of these results will require additional in vitro testing with other phenothiazines and in vivo testing with animal models of infection. Finally, CPZ has adverse effects on host defenses (2, 3, 5, 10), and these side effects will require careful consideration before any meaningful therapeutic uses develop.

ACKNOWLEDGMENTS

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LITERATURE CITED