Activity of Phenothiazines against Medically Important Yeasts

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Two phenothiazine compounds, trifluoperazine and chlorpromazine, inhibited growth in vitro of the five most common pathogenic yeasts, with MICs ranging from 10 to 40 μg/ml. Daily intraperitoneal injections of trifluoperazine (4 to 7 mg/kg of body weight) increased the survival of mice experimentally infected with Candida albicans or Cryptococcus neoformans. The potential use of these drugs against fungal meningitis is discussed.

The increasing occurrence of mycoses caused by opportunistic systemic infections (2, 3, 7) and the lack of effective and safe drugs (8) led to the search for new antifungal drugs with low toxicity. In previous studies in our laboratory, we investigated the effects and the mechanism of action of compounds from the phenothiazine group on the yeast Saccharomyces cerevisiae (4–6). It was found that trifluoperazine (TFP) and chlorpromazine (CPZ) cause a quick and massive K+ efflux leading to membrane hyperpolarization, Ca2+ influx, and inhibition of the plasma membrane H+ -ATPase. The results led to the suggestion that these substances, which are currently used as tranquilizers and in antipsychotic therapy (1), may be used as drugs against pathogenic yeasts in systemic infections. Since the phenothiazines accumulate in the central nervous system (1, 2), these drugs may be specifically effective against fungal meningitis and encephalitis. In the present study, we investigate the activity of TFP and CPZ against five medically important yeasts.

The yeasts used in the experiments were isolated from clinical specimens taken from different body sites of patients before treatment. Only one isolate per patient was studied. The yeasts were identified to the species level by current conventional methods (11). The isolates were maintained on Sabouraud dextrose agar until tested. In each in vitro experiment, at least five isolates of each species were tested. The mice used for in vivo experiments were Sabra white female mice 20 to 25 g in weight, and each experiment was repeated three times.

MICs were determined by the agar dilution method as described previously (10), with the exception that HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; 20 mM, pH 7.2) was included in the agar medium. For examining the pH effect, plates with acidic pH values were prepared by the addition of MES (4-morpholineethanesulfonic acid) at the required pH.

For the susceptibility test by the broth dilution method, yeast suspensions were prepared from overnight growth on Sabouraud dextrose agar at 30°C and added, at a density of 5 × 10^5 cells per ml, to test tubes each containing 10 ml of yeast nitrogen base (YNB) broth supplemented with glucose (100 mM), HEPES buffer (20 mM, pH 7.2), and the required concentration of the drug being tested. The loosely capped test tubes were incubated at 30°C in a shaker. Every 2 h, the optical densities at 530 nm were determined. MIC_{50} was the concentration of the drug at which the increase in the optical density at 530 nm after 8 h was less than 20% of that of the drug-free control. In these tubes, the broth remained visibly clear.

Infections in healthy female mice were produced by intraperitoneal injection (via the lateral tail vein) of 3 × 10^6 viable Cryptococcus neoformans cells or 3 × 10^5 viable Candida albicans cells suspended in 0.25 ml of sterile phosphate-buffered saline. The mice were placed into cages, with 7 to 10 mice in each group. Beginning 1 day postinfection, portions of TFP at the required concentration in 0.3 ml of sterile saline were injected daily intraperitoneally (i.p.) into the infected mice. In addition, two control groups of mice were tested. One group was injected with the yeast cells and was injected daily with 0.3 ml of sterile saline; a second control group was not infected with the yeast but was injected with the highest concentration of TFP used in the experiment. The survival of the mice was noted daily.

The five most common pathogenic yeasts were found by the agar dilution method to be susceptible to TFP and CPZ, with MICs for 50% of the strains ranging between 10 and 40 μg/ml (Table 1). The most susceptible yeast was C. neoformans, for which growth inhibition occurred at 10 μg/ml in 50% of the isolates examined. Most of the species showed similar susceptibility to TFP and CPZ.

For all of the species tested, except C. neoformans, MIC_{50} obtained by the broth dilution method was 50% of that obtained by the agar dilution method. Growth inhibition by TFP started with the initiation of the increase in the turbidity, usually 2 to 3 h after the beginning of the experiment.

Yeasts grown on YNB agar were found to be more susceptible to the drugs than were yeasts grown on Sabouraud dextrose agar. Addition of 7% sterile plasma to the YNB agar did not alter the MICs. The highest susceptibility on YNB agar was obtained at a pH range of 7.0 to 7.4 for all species. The MICs increased considerably with the decrease in medium pH.

Daily i.p. injection of TFP in doses ranging between 4.2 and 28 mg/kg of body weight prolonged the survival of mice infected with C. neoformans or C. albicans as compared with the nontreated infected control mice. For mice infected with C. neoformans (3 × 10^6 cells per ml), survival after 25 days was 1 of 10. Daily i.p. injections with 7 mg of TFP per kg of body weight increased the survival after 25 days to 7 of
TABLE 1. MICs of TFP and CPZ against various species of yeasts obtained by the agar dilution method

<table>
<thead>
<tr>
<th>Yeast tested</th>
<th>Drug</th>
<th>No. of isolates</th>
<th>Approx MIC&lt;sub&gt;90&lt;/sub&gt; (µg/ml)</th>
<th>MIC range (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>TFP</td>
<td>8</td>
<td>30</td>
<td>20-40</td>
</tr>
<tr>
<td></td>
<td>CPZ</td>
<td>7</td>
<td>25</td>
<td>20-35</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>TFP</td>
<td>9</td>
<td>35</td>
<td>25-40</td>
</tr>
<tr>
<td></td>
<td>CPZ</td>
<td>7</td>
<td>35</td>
<td>20-40</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>TFP</td>
<td>8</td>
<td>30</td>
<td>15-40</td>
</tr>
<tr>
<td></td>
<td>CPZ</td>
<td>5</td>
<td>35</td>
<td>10-40</td>
</tr>
<tr>
<td>Torulopsis glabrata</td>
<td>TFP</td>
<td>8</td>
<td>30</td>
<td>20-40</td>
</tr>
<tr>
<td></td>
<td>CPZ</td>
<td>7</td>
<td>35</td>
<td>10-40</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>TFP</td>
<td>6</td>
<td>10</td>
<td>10-35</td>
</tr>
<tr>
<td></td>
<td>CPZ</td>
<td>6</td>
<td>25</td>
<td>10-40</td>
</tr>
</tbody>
</table>

<sup>a</sup> MIC<sub>90</sub> denotes the lowest concentration of the drug which inhibited visible colony formation in 50% of the isolates.

10. For mice infected with C. albicans (3 × 10<sup>5</sup> viable cells per mouse), survival after 4 days was 1 of 7. Daily injections of 4.2 mg of TFP per kg of body weight increased survival after 4 days to 6 of 7. For both yeasts, the survival of the treated mice was significantly different from that of the untreated mice according to the Fisher exact test (P = 0.010 and 0.015, respectively). Increasing the doses of TFP up to 20 mg/kg of body weight did not improve the results.

Since both CPZ and TFP are currently used as pharmacological compounds, toxicity was not investigated extensively. In one experiment, we observed that a single i.p. injection dose of 283 µg of TFP per kg of body weight into noninfected mice caused a visible tranquilizing effect 1 day postinjection. There was no effect on their survival. Daily i.p. injections of 56 µg of TFP per kg of body weight into noninfected mice over 30 days did not affect their survival and had a mild tranquilizing effect on these mice.

Opportunistic fungal infections represent an increasing threat, mainly to immunosuppressed patients (2, 3, 15). Yet the few drugs available have limited efficiency or moderate toxicity (8, 12, 13). The problem is particularly severe in fungal meningitis since amphotericin B, the most efficient antifungal drug available, poorly penetrates the blood-brain barrier (8).

The phenothiazines are a group of potent pharmacological agents with neuroleptic, antiemetic, antihistaminic, anticholinergic, and sedative activities (1, 14). Their main pharmacological effect is determined by variations in the chemical structure of the side chain at position 10 of the phenothiazine ring. In the present study, two of these compounds, TFP and CPZ, inhibited growth in vitro of five pathogenic yeasts.

In mice infected experimentally with C. neoformans or C. albicans, daily injections of TFP caused an increase in the survival time. The lower doses used in these in vivo experiments were of the same order of magnitude as the therapeutic doses of these substances. Since the phenothiazines accumulate in the central nervous system and since their concentration in the brain may reach 10 times the concentration in serum (9), these substances may be particularly suitable, after additional research, for treatment of fungal meningitis and encephalitis caused by pathogens, mainly those resistant to flucytosine, since a high frequency of resistance to flucytosine has been reported (13).

It was recently suggested that phenothiazines such as CPZ, TFP, and others may be given to cancer patients to reduce the chemotherapy-induced emesis (14). It would be beneficial to determine whether these drugs, when administered as antiemetics, also have prophylactic effects in fungal infections, which are common in cancer patients (2).

The yeast S. cerevisiae has a susceptibility to TFP similar to that of C. albicans. The mechanism of action of TFP on S. cerevisiae has been investigated recently in our laboratory (4-6). It was found that TFP affects yeast cell membranes within 30 min of addition by causing K<sup>+</sup> efflux, membrane hyperpolarization, increased Ca<sup>2+</sup> influx, and inhibition of the plasma membrane H<sup>+</sup>-ATPase. The membrane-damaging effects were most pronounced above pH 7.0 (6). In addition to these effects, it was found that low concentrations of TFP, which did not cause membrane damage, caused an arrest of cell growth at specific points of the cell cycle (Y. Eilam and D. Chernichovsky, unpublished results). Thus, the mechanism of action of the phenothiazine on susceptible yeasts involved both membrane damage and interference with the cell cycle.

There is a potential for using phenothiazines as drugs against systemic yeast infections and particularly in the treatment of fungal meningitis, but further investigation is required.

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LITERATURE CITED


