Inhibitory Effects of Seven Organosulphur Compounds on Clinical Isolates of Candida Species In Vitro*

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ABSTRACT

Thirty clinical isolates of Candida albicans and 10 other Candida species were tested for susceptibility to 6 substituted dithiocarbamates and one dimercaptosuccinate. Dimethyldithiocarbamate, sodium pyrrolidine dithiocarbamate, and sodium diethyldithiocarbamate showed dose-dependent antifungal activity which was partially reversed by the addition of zinc, copper, or iron sulfate with greatest reversal at 2:1 metal to dithiocarbamate molar ratio. Anaerobiosis also interfered with dithiocarbamate antifungal activity.

Introduction

Candida albicans is the most commonly encountered fungal pathogen in clinical practice and presents a serious problem as a cause of superficial or systemic infection in patients with suppressed or deficient immune response. Antifungal drugs such as amphotericin-B or ketoconazole are not always effective in controlling candidiasis, especially in disseminated cases. Amphotericin B is associated with a number of undesirable side effects of which nephrotoxicity is most significant. Toxicity limits its use or necessitates premature discontinuation of therapy. Patients with normal immune status may respond well to treatment with ketoconazole, but immunocompromised patients with disseminated candidiasis often show less impressive responses. Thus, new therapies for Candida should be sought.

This study evaluated the anticandidal effects of several structural analogs of dithiocarbamates in vitro. Diethyldithiocarbamate has previously shown antifungal activity against 3 different strains of Candida albicans, and
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Figure 1. Seven organosulfur compounds were tested against 40 clinical isolates of Candida. Bars indicate average ± standard deviation of zone size for each compound. Sodium dimethyldithiocarbamate (DMDTC), sodium pyrrolidine dithiocarbamate (PYRDTC), and sodium diethyldithiocarbamate (DDTC) showed substantial antifungal effect. Little antifungal effect was shown by compounds sodium monomethyldithiocarbamate (MMDTC), sodium monoethyldithiocarbamate (MEDTC), sodium N-methyl-D-glucamine dithiocarbamate (NMG-DTC), and monoisoamyl meso-2,3-dimercaptosuccinate (Mi-ADMS).

plans were made to evaluate additional analogs of dithiocarbamates against larger numbers of clinical Candida isolates.

Materials and Methods

Seven organosulfur compounds including six dithiocarbamates and one dimercaptosuccinate were used in this study. The chemicals were purchased directly from scientific companies: sodium diethyldithiocarbamate (DDTC),* and sodium dimethyldithiocarbamate (DMDTC).† Sodium pyrrolidine dithiocarbamate (PYRDTC), sodium monoethyldithiocarbamate (MEDTC),‡ sodium monomethyldithiocarbamate (MMDTC), monoisoamyl meso-2,3-dimercaptosuccinate (Mi-ADMS) and sodium N-methyl-D-glucamine dithiocarbamate (NMG-DTC) were synthesized by two of the authors.⁵,⁶ Methods of synthesis, purification and analysis have been reported elsewhere.⁶ All compounds were stored at -20°C until used. Forty clinical isolates of Candida were obtained from the clinical microbiology laboratory at Saint Mary’s Hospital, Huntington, WV. No attempt was made to record the site of infection or severity of illness with these organisms. Thirty of the 40 isolates were of Candida albicans and 10 remaining isolates were of Candida species.

The antifungal property of these organosulfur compounds was tested by an agar dilution technique, on Sabouraud’s dextrose agar.⁷ Candida isolates were grown overnight at 37°C in Sabouraud’s broth and diluted to A₅₇₀ 0.1 to 0.2. Agar plates were streaked with sterile swabs moistened with the starter culture to produce a uniform lawn. Blank 7 mm sterile filter paper discs were dropped onto the inoculated plates and each disc was loaded with 20 μg of the test compounds dissolved in saline (unless otherwise noted). Plates were incu-

† Eastman Kodak Co., Rochester, N.Y.
bated at 37°C for 24 hours and growth inhibition zones were measured with vernier calipers. Zone sizes greater than or equal to 14 mm indicated sensitivity to the compound. Inhibitory activity of DMDTC when combined with metal salts: ZnSO₄ · 7H₂O, CuSO₄ · 5H₂O, or FeSO₄ · 7H₂O, was further evaluated. These metal salts were mixed with 0.01M concentrations of DMDTC to produce several (0.25 to 2.00) metal: DMDTC ratios. These mixtures were applied to discs and placed on lawns of Candida albicans.

The effect of DMDTC under anaerobic conditions on two randomly selected strains of Candida albicans was determined. These were grown overnight at 37°C in Sabouraud's broth, and their susceptibility against DMDTC was tested by agar dilution in the presence and absence of oxygen. Plates were evaluated at 24 hours.

Results

Six dithiocarbamates and one dimercaptosuccinate (20 μg/disc) were tested for their ability to inhibit 40 clinical strains of Candida. Average zone size is shown in figure 1, which indicates that three of these compounds, DDTC, DMDTC, and PYRDTC, showed a significant (>14 mm zones) inhibitory effect with DDTC appearing less active (2 of 40 strains provided zones <14 mm against DDTC) than DMDTC and PYRDTC. However, the experiment summarized by figure 1 did not compare equimolar concentrations of the 7 organosulfur compounds. Therefore, 10 randomly selected strains of Candida albicans were retested with discs containing 20 μl of 10⁻⁵M, 10⁻⁴M and 10⁻³M concentrations of DDTC, DMDTC and PYRDTC. As shown in figure 2, the three dithiocarbamates demonstrated similar susceptibility at 10⁻⁴M, but DDTC appeared less active than the other two.
compounds at the highest and lowest concentrations. The fungal inhibitory effect was dose-dependent for all three compounds tested.

The mechanism of anticandidal activity is not known but may involve the metal combining capacity of these dithiocarbamates as described in previous reports.9 This mechanism was evaluated by measuring the inhibitory activity of DMDTC when combined with metal salts. The effect of these salts was determined by comparing zone sizes with the metals to the zone sizes without added metal. It is indicated in figure 3 that all the metals tested decreased the antifungal effect with the greatest reversal at a 2:1 metal:DMDTC molar ratio.

Reversal of inhibition was also observed with FeCl₂. 4 H₂O. These data support our hypothesis that the dithiocarbamates exhibit their antifungal action through the binding of metals critical to the growth of the organisms. The addition of metals may have chelated the dithiocarbamates thus decreasing their availability to chelate intracellular metals. However, incomplete reversal by metals suggested the existence of an additional inhibitory mechanism. Under anaerobic conditions the antifungal effect of DMDTC was decreased by 52%. There was an appearance of double zones of inhibition on some agar dilution plates.

**Discussion**

Dithiocarbamates are known inhibitors of superoxide dismutase.4 Inhibition of this system may allow the accumulation of free radicals within the fungi causing oxidative damage to fungal structures. Theoretically, under anaerobic conditions superoxide should not accumulate, obviating the need for superoxide dismutase and thereby diminishing the apparent effect of dithiocarbamate on the fungi. The incomplete reversal of inhibition may have resulted from a second (metal-based) mechanism of antifungal activity. This is also consis-
tent with dual mechanisms of inhibition: (1) chelation of critical fungal metals and (2) oxidative damage to fungi.

Dithiocarbamates could have potential clinical use in the treatment of fungal infections, if they prove to have appropriate in vivo safety as well as efficacy. The available information on toxicological properties of the dithiocarbamates and related thiuram sulfides shows these compounds to be relatively nontoxic. Dithiocarbamates are used as agricultural fungicides with a low order of acute toxicity to animals and humans. Since DDTC and its dimer disulfiram (Antabuse®) have been used clinically for other purposes some safety questions have already been addressed. Sodium diethylthiocarbamate is an approved orphan drug for the treatment of nickel toxicity, while Antabuse® has previously been used for years in the treatment of chronic alcoholism. Contact dermatitis and a disulfiram-like response with ingested alcohol are among the few adverse effects seen in human usage. It is possible that chemical analogs of DDTC with optimal risk-benefit profiles may be developed as antifungal agents for monotherapy or may be used to augment the activity of amphotericin-B or other approved antifungal agents.

References