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Disulfiram Inhibits the In Vitro Growth of Methicillin-Resistant *Staphylococcus aureus*

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Several antibiotics have disulfiram-like effects; we evaluated disulfiram for its antibiotic-like effects. Disulfiram inhibited the in vitro growth of methicillin-resistant *Staphylococcus aureus*, with an MIC of 1.33 μg/ml, but was not effective against members of the family *Enterobacteriaceae* or *Pseudomonas* species.

The medical management of chronic alcohol abuse frequently includes treatment with disulfiram (DSF) as an aid to maintaining long-term sobriety. A patient who consumes an alcoholic beverage during treatment with DSF will promptly experience the subjectively unpleasant disulfiram-ethanol reaction, which includes palpitations, malaise, flushing, nausea, and vomiting. The molecular basis of this interaction has been ascribed to the noncompetitive inhibition of aldehyde dehydrogenase by DSF, so that the ingestion of ethanol is followed by the accumulation of its first metabolite, acetaldehyde, in toxic quantities (8, 10, 13, 16). However, the disulfiram-ethanol reaction might also be mediated by other enzymes, including dopamine β-hydroxylase (19) and alcohol dehydrogenase (2), which are also inhibited by DSF.

It is now well known that patients treated with a number of antimicrobial agents are also at risk for a clinically similar reaction should they consume any alcohol. Disulfiram-like interactions with ethanol have been observed during treatments with cephalosporins, metronidazole, quinacrine, chloramphenicol, and moxalactam (6, 12, 25).

While it is apparent that these antibiotics can simulate the clinical effects of DSF, there is now evidence to suggest that the converse might also be true, i.e., that DSF may in some cases act as an antibiotic. Scheibel et al. (24) found that DSF inhibited the growth of the human malaria parasite *Plasmodium falciparum* in vitro. The major breakdown product of DSF in vivo is diethyldithiocarbamate (7) (DDC), which has activity against *Pityrosporum canis* and inhibits the growth of ear mites (*Otodectes cynotis*) in cats and dogs (17). DDC may also act as an immunomodulator and viricide and may clinically benefit patients suffering from infection with human immunodeficiency virus (14, 23). Taylor et al. (26) recently reported that DDC inhibits the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) and several other microorganisms in vitro. These findings prompted us to investigate the effects of DSF on bacterial growth, and we present here new evidence that DSF inhibits the growth of MRSA in vitro.

Preparation of DSF disks. Blank paper disks (diameter, approximately 0.64 cm) (1599-33; Difco Laboratories, Detroit, Mich.) were dried after being loaded with a freshly prepared solution of DSF (Abbott, North Chicago, Ill.) in 95% ethanol. Fresh disks containing 5, 10, 100, or 200 μg of DSF were prepared each day before use. Control disks (prepared similarly with 95% ethanol containing no DSF) were included in each test run.

Preparation of bacterial cultures. Organisms were obtained from stock cultures maintained on cystine-tryptic agar by the Clinical Microbiology Laboratory of St. Vincent's Medical Center of Richmond. *S. aureus* isolates were identified by colonial morphology, catalase production, and latex particle agglutination (Staphaurex; Wellcome Diagnostics, Research Triangle Park, N.C.). A microtiter identification system was used to identify members of the family *Enterobacteriaceae* and *Pseudomonas* species to the species level and to determine antibiotic susceptibility (MicroScan; Baxter Healthcare Corp., West Sacramento, Calif.). Each organism was subcultured from cystine-tryptic agar onto tryptic soy agar containing 5% sheep blood and incubated aerobically at 35°C for 18 h. MRSA was defined as an organism for which the oxacillin MIC was greater than or equal to 4.0 mg/ml and which showed resistance to cephalosporins and susceptibility to vancomycin (21). Twenty different strains of *Staphylococcus aureus* were tested, and 10 of these strains were MRSA.

Incubation of bacteria with DSF. Antimicrobial disk susceptibility tests were performed by standard methods (21). A standardized inoculum was swabbed onto commercially prepared 150-mm Mueller-Hinton plates supplemented with 25 mg of magnesium and 50 mg of calcium per ml (BBL Microbiology Systems, Cockeysville, Md.). One disk of each concentration of DSF and one control disk were placed on the surface of the agar plates. Ten plates were prepared for each organism. After aerobic incubation at 35°C for 18 to 24 h, the diameter of the zone of inhibition was measured with calipers.

Determination of MIC of DSF. Since DSF is poorly soluble in water, MICs were determined by using serial dilutions of a saturated solution. DSF was sonicated in 0.85% saline and then centrifuged at 1,300 × *g* for 60 min; the concentration of DSF in the saturated supernatant was determined by high-pressure liquid chromatography assay (18). Broth macrodilution susceptibility tests were performed by standard methods (20). Serial twofold dilutions of DSF solution were prepared in Mueller-Hinton broth supplemented with 25 mg of magnesium and 50 mg of calcium per ml. A standard inoculum (5×10^4 CFU) was added to each tube. All tubes were incubated for 18 h at 35°C and then examined for turbidity by comparison with the growth control tube (con-

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taining no antimicrobial agent). The MIC was defined as the lowest concentration of DSF which inhibited visible growth.

Incubation of bacteria with DSF. Growth of MRSA was visibly inhibited by the disks impregnated with DSF, whereas there was no zone of inhibition around the ethanol control disks. The size of the zone of inhibition did not change significantly with the amount of DSF in the disk: mean zone diameter was 14.0 mm (standard deviation, 1.93) with 5 μg of DSF and 14.6 mm (standard deviation, 1.69) with 200 μg of DSF (not significant by the two-tailed unpaired *t* test). DSF did not visibly inhibit the growth of members of the family *Enterobacteriaceae* or *Pseudomonas* species.

MIC of DSF. The concentration of DSF in the saturated aqueous solution was found to be 2.66 μg/ml by high-pressure liquid chromatography assay. In serial dilutions, the MIC of DSF for all isolates of MRSA was 1.33 μg/ml.

DSF was clearly seen to inhibit the in vitro growth of MRSA. When antibiotics such as penicillins and cephalosporins are incubated with MRSA on agar, the size of the zone of inhibition usually varies with the quantity of drug in the disk. No such gradation of response was observed in this study; similar inhibition zone diameters were observed with disks containing 5 to 200 μg of DSF. The low solubility of DSF in water may account for this finding: the quantity of drug in the lowest concentration disk may have been sufficient to saturate the aqueous phase of the surrounding agar.

The MIC of DSF for MRSA was found to be 1.33 μg/ml. This in vitro finding cannot be readily applied to levels in serum, nor can inferences be made about clinical efficacy in humans, principally because a number of investigators have not been able to measure the concentration of unchanged DSF in the serum of subjects treated with the oral drug (11, 18, 22). DSF is unstable in the blood, where it is rapidly and completely converted to two molecules of DDC by the action of serum albumin and erythrocyte enzymes (1, 3). Although Das Gupta demonstrated that aqueous suspensions of DSF were chemically stable for at least 295 days (4), it is possible that organic components of the culture medium catalyzed breakdown of the drug to form DDC or other active metabolites.

There are several possible mechanisms by which DSF might have inhibited the in vitro growth of MRSA. DSF might have been metabolized by the bacteria to form DDC, which is also an active inhibitor of MRSA growth (26). In addition, DSF and a number of its metabolites might inhibit bacterial growth by the chelation of metallic ions (9) or by the inhibition of enzymes, including aldehyde dehydrogenase (13, 16), alcohol dehydrogenase (2), lactate dehydrogenase (15), and dopamine β-hydroxylase (19). Enzymic inhibition by DSF has been ascribed to irreversible disulfide interchange reactions with thiol groups (5). Any or all of these mechanisms may have been involved, but further studies are required to determine the specific mechanism by which DSF inhibits the growth of MRSA.

The inhibitory effects of DSF on the growth of MRSA have not been reported previously to our knowledge. The findings of this pilot study should be interpreted with caution and should not be extended to clinical applications until the effects of DSF have been rigorously evaluated in animal models of infection. However, in view of the growing problem of MRSA infection in hospitalized patients, we suggest that DSF merits further investigation as a potential new antibiotic agent.

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