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Antibacterial Activity of Niridazole against Salmonellae

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Fifty-six strains, representing eight species of salmonellae of diverse geographic origin and possessing a variety of antimicrobial resistance profiles, were tested for susceptibility to niridazole by the agar dilution method. Calculated MICs for 50 and 90% of strains were 4.8 and 16.0 mg/liter, respectively, with a susceptibility range of 0.25 to 32 mg/liter. No obvious species differences were noted. Niridazole was found to be rapidly and powerfully bactericidal. No significant difference was detected between MICs and MBCs. Except for a strain-dependent effect with a subset of multiply resistant salmonella isolates, no inoculum effect was demonstrated.

Chronic salmonella bacteremia is a regular feature of hepatosplenic schistosomiasis, and niridazole is active against both of the associated pathogens (5). The attractiveness of a "two birds with one stone" approach with niridazole, and the limited available information on the susceptibility of salmonellae to niridazole, prompted the present study.

Strains. Fifty-six strains, representing eight species of salmonella including *S. typhimurium*, *S. enteritidis*, and *S. typhi* of North American, Asian, South American and Indian origins, and possessing a variety of antimicrobial resistance profiles, were selected for testing.

Preparation of inoculum. All strains had been maintained at −70°C and were subcultured to Mueller-Hinton agar before test. Isolated colonies were inoculated into Mueller-Hinton broth and incubated at 37°C for 6 h. The turbidity of the suspension was then adjusted to an optical density reading of 0.8 at 650 nm, using a Coleman Junior IIA spectrophotometer (The Perkin-Elmer Corp., Norwalk, Conn.). This was equivalent to a density of 3.5×10^8 cells per ml as determined by viable count. Dilutions were then prepared so that the final inocula with a Steers multiple replicator apparatus were 10^3, 10^4, 10^5, and 10^6 organisms per prong.

Susceptibility. (i) MICs. Niridazole (kindly supplied by CIBA-GEIGY Corp., Summit, N.J.) was dissolved in N, *N*-dimethylformamide and further diluted in distilled water. Susceptibility was determined by an agar dilution method, using a multiple replicator and Mueller-Hinton agar containing doubling concentrations of niridazole ranging from 0.125 to 64 mg/liter. Plates were incubated aerobically at 37°C for 16 to 18 h. Susceptibility was indicated by the absence of growth or the presence of fewer than five colonies at the point of inoculation. Control strains of *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25293, and *Escherichia coli* ATCC 25922 were included for reference.

(ii) MBCs. The MBCs of 10 salmonella strains representing isolates with low, intermediate, and high MICs were selected for determination of the MBC in duplicate by the broth tube dilution method. Mueller-Hinton broth (2.0 ml) containing niridazole concentrations from 0.125 to 256 mg/liter was inoculated with 1.0×10^6 to 1.5×10^6 organisms, and the tubes were incubated at 37°C in heating blocks. At 1, 2, 4, 6, and 24 h, 0.01-ml aliquots were sampled, and colony counts were performed on Mueller-Hinton agar after a further 24-h incubation at 37°C. The MBC was defined as the lowest antibiotic concentration producing a minimum of a 99.9% reduction in viable count of the initial inoculum.

The MIC_{50} and MIC_{90} (concentrations that inhibited 50 and 90% of the strains, respectively) were 4.8 and 16.0 mg/liter as calculated by the Reed and Muench method (7). Individual susceptibilities ranged from 0.25 to 32 mg/liter. No obvious species differences were noted. Control strain MICs were in the expected range (2). The MBCs for the 10 representative salmonella strains were equal to or only one twofold dilution higher than the respective MICs. Niridazole was also found to be rapidly and powerfully bactericidal. A decrease of 2 logs in the viable count was observed within 2 h of exposure to the drug, and a further 2-log decrease was noted at the end of 4 h. Except for 2 strains of the 10 tested, inoculum size had no significant effect on the MICs (within one twofold dilution). These two isolates, which had niridazole MICs in the upper end of the range, had a multiply-resistant antibiotic profile (ampicillin, tetracycline, chloramphenicol, trimethoprim, sulfamethoxazole, and gentamicin) and exhibited fourfold increases in the MIC with increasing inoculum size (10^4 to 10^6). To determine if this was a strain characteristic, an additional eight related strains with the same antibiogram were retested. Irrespective of the basic MIC, all isolates with this antibiotic resistance pattern showed a reproducible fourfold increase in MIC with various inoculum sizes.

The MICs obtained in the above agar dilution study are in line with those obtained by earlier disk and tube dilution methods (9) and with the limited, current agar dilution data (2). Concentrations of niridazole close to the MIC were rapidly and intensely bactericidal in a fashion similar to that reported for a single strain of *E. coli* (4). Previous studies on the activity of niridazole against salmonellae did not examine an inoculum effect. No significant inoculum effect was noted in the present survey for the majority of isolates, and even in the multiply-resistant subset of strains where an effect was observed, it was not pronounced.

Recent studies on the pharmacokinetics of oral niridazole and its metabolites after 15-mg/kg doses revealed 1-h serum levels of niridazole of only 0.4 mg/liter (8)—10-fold less than the MIC_{50} for salmonellae. Despite this, there is considerable evidence that niridazole is effective in experimental systemic salmonella infection (1, 3), and complementary data exists suggesting a role for the drug in the treatment of

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human schistosomiasis associated with salmonella bacteremia (6).

It is possible that the reconciliation of the in vitro and in vivo performances of niridazole will be uncovered when more detailed information on the antimicrobial activity and pharmacokinetics of its several metabolites becomes available (5, 8).

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