Effect of Oral Niridazole Treatment on Some Bacterial Infections in Mice

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Treatment of specific-pathogen-free CD-1 mice with oral doses of 10 or 100 mg of niridazole per kg of body weight given 24 h before challenge and then every other day for up to 15 days altered the growth curves for Listeria monocytogenes, Mycobacterium bovis (BCG Montreal), M. tuberculosis H37Rv, and Salmonella enteritidis seen in the livers and spleens of the treated animals. Niridazole in an oral dosage of 10 mg/kg reduced (but did not eliminate) tuberculin hypersensitivity in the mycobacteria-infected mice. Both delayed hypersensitivity and antimonycobacterial resistance quickly returned to normal levels once the drug treatment was stopped. Niridazole treatment reduced the growth of S. enteritidis in both intravenously and intragastrically challenged mice; this seemed to be due to the antibacterial action of the drug on the salmonellae both in vitro and in vivo.

Niridazole is a major antischistosomal drug that has been tested in several human trials with highly encouraging results (1, 14). The drug acts as an anti-inflammatory agent (3) and strongly suppresses delayed-type hypersensitivity responses to schistosome egg proteins in the liver and lungs (13, 17). Schistosomiasis is known to aggravate and prolong enteric and urinary salmonella infections in humans (11, 14, 16). Niridazole treatment, by depressing the ability of the host to mount a cellular immune response to the bacteria-sensitizing antigens (3), could increase the severity of the concurrent microbial infection in the schistosomal patient. Watson (18) recently reported that systemic niridazole treatment of Salmonella-infected mice did not exacerbate the disease. On the contrary, treatment of Salmonella typhimurium-infected mice with large doses of the drug actually reduced the level of splenic involvement compared with that of the normal controls. This unexpected effect seems to be due to the antibacterial activity shown by niridazole against S. typhimurium. When tested in vitro, the drug was growth inhibitory at a high dilution, suggesting that it was acting directly on the bacterial population in vivo (18). The antibacterial and anti-inflammatory effects of this drug could thus be expected to offset each other; the results of this study suggest that this is indeed the case.

MATERIALS AND METHODS
Organisms. S. enteritidis NCTC 5694, S. gallinarum NCTC 9240, and S. typhimurium C5 have been described previously (2, 6). S. typhi Ty2 and a hybrid of S. typhi-typhimurium (10) were kindly supplied by E. M. Johnson, Walter Reed Army Research Institute, Washington, D.C. Listeria monocytogenes, Mycobacterium bovis (BCG Montreal), and M. tuberculosis H37Rv were grown and stored as described elsewhere (4, 8, 9).

Drug treatment. Niridazole (Ambilhar) was generously donated by C. A. Brownly of CIBA Pharmaceuticals, Summit, N.J. Because the drug has a relatively low solubility in water, 10 mg (dry weight) had to be suspended in 1 ml of distilled water and shaken at 37 C for 2 h. The continuously stirred suspension was then introduced into specific-pathogen-free CD-1 mice intragastrically (5). Individual dosage rates corresponded to 10 or 100 mg of niridazole per kg of body weight. The drug was administered 24 h before the microbial challenge; in most of the mice, repeated doses were given every other day for up to 15 days. Control mice received 0.2 ml of sterile distilled water given orally every other day. Details of the infection methods, bacterial enumeration, tuberculin hypersensitivity measurements, and minimal inhibitory concentration (MIC) determinations are given in the accompanying paper (4).

RESULTS
L. monocytogenes infection. Inoculation of mice with a mean lethal dose of L. monocytogenes was followed by a period of logarithmic growth in both the liver and spleen with a sharp decline in viable counts beginning on day 2 (Fig. 1). Prior treatment of the mice with either 10 or 100 mg of niridazole per kg of body weight and then with repeated doses given every other day reduced the size of the 2-day peak but did not greatly affect the immune response that
developed to the infecting bacteria. A single dose of 100 mg of drug per kg, administered 1 day before challenge, reduced the size of the liver and spleen counts 10-fold compared with the controls but could not affect the subsequent immune response (Fig. 1). None of the treated mice died, but two of five controls were dead by day 6.

**Mycobacterial infections.** Treatment of normal mice with 10 mg of niridazole per kg every other day for 15 days had little effect on the growth of either the BCG or H37Rv populations in the lungs, liver, or spleen compared with the control growth curves (shown in the accompanying paper) (Fig. 2 and 3; reference 4). An immune response was normally seen about day 14 in untreated mice (7), but there was no sign of an immune decline in viable counts for *M. tuberculosis* H37Rv (Fig. 2). The level of layed hypersensitivity observed on day 14 was also 50% lower than expected, but, at the niridazole concentrations used in this study, tuberculin hypersensitivity was never completely suppressed. After the cessation of drug treatment on the 14th day of the mycobacterial infection, there was an obvious increase in tuberculin hypersensitivity over the next 7 days, and this event coincided with the emerging immune decline in the bacterial counts seen in the spleens and lungs of these mice (Fig. 2). None of the infected mice died during the experimental period.

**Salmonella infections.** Treatment of mice with niridazole at either 10 or 100 mg per kg of body weight, given every other day, markedly depressed the growth of the *S. enteritidis* populations in both the liver and spleen. The effectiveness of a 1.0-mg dose per kg of body weight was debatable, but there was no question that the 10-mg and particularly the 100-mg dose per kg reduced growth of the *Salmonella* population significantly (*P* < 0.01) when compared with that seen in the control animals after 4 to 6 days (Fig. 3). A single dose of 100 mg of niridazole per kg given 24 h before challenge, however, had much less effect on the subsequent growth of the salmonellae. Treatment of the mice with 100 mg/kg starting 24 h or more after the infection resulted in little change in the course of the

**FIG. 1.** (Bottom) Growth of intravenous inocula of *L. monocytogenes* in mice treated with a single dose of niridazole (100 mg/kg) or repeated doses given every other day (broken lines). Symbols: O, liver; □, spleen. Each point represents an average of five determinations. The vertical bars represent the standard error of the mean. (Top) Growth of *L. monocytogenes* in mice receiving repeated doses of 10 mg of niridazole per kg every other day (solid lines). The dotted lines represent the growth of the challenge organism in normal controls.

**FIG. 2.** Growth of *M. tuberculosis* H37Rv (bottom) or BCG Montreal (top) after intravenous challenge in mice receiving niridazole (10 mg/kg) every other day for 15 days. The normal control curves are shown in the accompanying paper (4). Symbols: ■, spleen; ●, liver; ▲, lungs. The histograms represent the 24-h foot swelling 24 h after 2.5 μg of purified protein derivative was injected into a hind footpad. Increases of 1.8 units (0.18 mm) or more were significant at the 1% level.
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Niridazole had a low level of solubility in tryptose soy broth (250 μg/ml). Although there was some variation in the MICs obtained with the different salmonellae tested (Table 1), growth of both S. typhi Ty2 and S. enteritidis was inhibited by as little as 1 to 2 μg of niridazole per ml of broth. On the other hand, L. monocytogenes required 30 times this dose to be completely inhibited, and growth of M. bovis (BCG) was unaffected by 250 μg of niridazole per ml (Table 1). The MIC data clearly explain the relatively poor growth seen in the S. enteritidis-challenged mice receiving repeated doses of 100 mg of niridazole per kg of body weight (Fig. 4).

**DISCUSSION**

Niridazole has a powerful antischistosomal effect even when tested at very small doses. The immunosuppressive effects of the drug (12) were expected to have some influence on the development of a concurrent salmonellosis. The present study makes it clear, however, that any immunosuppressive action (at least at dosages up to 100 mg/kg) was countered by a considerable antibacterial activity by niridazole on the Salmonella population in vivo. Watson (18) essentially free of salmonellae by day 12.

**Antimicrobial activity of niridazole in vitro.** Niridazole had a low level of solubility in tryptose soy broth (250 μg/ml). Although there was some variation in the MICs obtained with the different salmonellae tested (Table 1), growth of both S. typhi Ty2 and S. enteritidis was inhibited by as little as 1 to 2 μg of niridazole per ml of broth. On the other hand, L. monocytogenes required 30 times this dose to be completely inhibited, and growth of M. bovis (BCG) was unaffected by 250 μg of niridazole per ml (Table 1). The MIC data clearly explain the relatively poor growth seen in the S. enteritidis-challenged mice receiving repeated doses of 100 mg of niridazole per kg of body weight (Fig. 4).

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**TABLE 1.** Niridazole MIC determinations on various bacterial cultures

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. enteritidis 5694</td>
<td>1-2</td>
</tr>
<tr>
<td>S. gallinarum 9240</td>
<td>16</td>
</tr>
<tr>
<td>S. typhimurium C5</td>
<td>8</td>
</tr>
<tr>
<td>S. typhi Ty2</td>
<td>1</td>
</tr>
<tr>
<td>S. typhi-typhimurium #42</td>
<td>8</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>32</td>
</tr>
<tr>
<td>M. bovis (BCG)</td>
<td>&gt;250</td>
</tr>
<tr>
<td>M. tuberculosis H37Rv</td>
<td>&gt;250</td>
</tr>
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* Determined in 7H9 broth after 21 days.
reported greater efficacy when the drug was introduced parenterally into the host, stating that oral treatment was much less effective against an intraperitoneal challenge with virulent *S. typhimurium*. In the present study, intragastric inoculation of the drug induced excellent responses in vivo against both intravenous and intragastric *S. enteritidis* challenges in the CD-1 mice. Watson (18) also reported MIC values of 4 to 8 µg of niridazole for *S. typhimurium*, which agree very well with the values shown for this organism in Table 1. *S. typhi* and *S. enteritidis* appear to be even more susceptible to the drug, and it seems likely that niridazole treatment of patients suffering from schistosomiasis and enteric fever should gain more benefit than harm from oral treatment with this drug. The in vitro susceptibility of *S. enteritidis* 5694 to 1 µg of drug per ml reaffirms earlier suggestions (18) that niridazole may have clinical activity in the treatment of gastroenteritis and enteric fever in man.

Niridazole seemed to depress the level of tuberculin hypersensitivity seen on day 14, but by day 21 (6 days after stopping treatment) levels of hypersensitivity were back to those seen in the normal controls. The disappointingly small effect of niridazole treatment on the level of cellular hypersensitivity compared with that reported by others (12, 13) may have been due to differences in the dosages used. Attempts to prolong immunosuppression by the use of larger doses of drug over the first 15-day period of the infection were largely unsuccessful, although it is apparent that the normal mouse can tolerate doses of up to 600 mg per kg of body weight (18). There seemed little point, however, in pursuing the present study into dosage ranges beyond those normally used in human trials (1, 15).

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