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SALMONELLA TYPHIMURIUM INFECTION IN MICE TREATED WITH NIRIDAZOLE

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THE introduction of niridazole {1-(5-nitro-2-thiazolyl)-2-imidizolidinone} for treatment of schistosomiasis and amoebiasis was followed by a demonstration of its antibacterial properties. Neves *et al.* (1966) demonstrated activity against strains of salmonella both *in vitro* and *in vivo*. Ten patients with chronic systemic salmonellosis and associated schistosomiasis responded well to treatment. Culture from blood, stools and urine became negative either during or immediately after therapy. Salmonella strains involved in this series included *S. typhi*, *S. paratyphi A*, *S. montevideo*, *S. london*, *S. choleraesuis* var. *kunzendorf*, *S. paratyphi C*, *S. anatum*, *S. panama* and *S. newport*.

In view of these observations an investigation was undertaken of the effect of niridazole in mice experimentally infected with *S. typhimurium*. A study was also carried out of the effect of the drug *in vitro* against a number of different salmonella strains.

MATERIALS AND METHODS

Salmonella strains. Both fresh isolates and stock laboratory strains were used. Single colonies were picked off nutrient agar plates and subcultured in nutrient broth. Two further subcultures in broth were made before *in-vitro* or *in-vivo* testing.

Sensitivity tests. Pure crystalline niridazole in sterile distilled water was agitated continuously for 4 hr at 37°C since the upper limit of solubility is of the order of only 200 µg per ml. The saturated solution was filtered through Whatman no. 1 paper either at room temperature or at 37°C; 2 ml of this fluid were then added to 100 filter-paper disks (diam. 6.25 mm). Each disk contained approximately 4 µg. They were applied wet to the surface of nutrient agar plates that had been spread with three drops of a 6-hr culture of the appropriate organism, and then dried for 20 min. at 37°C.

The minimum inhibitory concentrations were determined by a standard tube dilution method in nutrient broth in 2.5-ml amounts. The inoculum consisted of one drop of a 16-hr culture in broth. The results were read after overnight incubation at 37°C.

In-vivo tests. Adult white mice (20–25 g weight) were used. Infection was by intra-peritoneal injection of 0.1-ml volumes of suspensions prepared from agar slope cultures

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washed off in sterile buffered 0.85 per cent. saline (pH 7.2). The inoculum contained approximately 1.5×10^4 organisms. Two strains were employed, designated as strain no. 8 and strain no. 12 by their laboratory accession numbers. Strain no. 12 was the more virulent and preliminary studies gave LD50 doses of approximately 2×10^4 and 8×10^3 organisms respectively, when deaths were recorded over a period of 21 days.

Treatment. Niridazole was administered according to the protocols given below. In addition to intraperitoneal and subcutaneous injection, several mice were given the drug by mouth, but this procedure was unsatisfactory and it was consequently abandoned. All the mice were observed daily and the deaths were recorded for 21 days.

Spleen counts. The survivors were killed on the 21st day and each spleen was removed aseptically and homogenised in 4 ml of nutrient broth in a tissue-grinder with sterile sand. Suitable dilutions in sterile saline with 5 per cent. broth were then plated in 0.02-ml volumes on nutrient agar plates according to the technique of Miles, Misra and Irwin (1938). Organisms were identified as *S. typhimurium* and the number per spleen was calculated.

TABLE I
Niridazole sensitivity of salmonella by the disk method

Organisms tested		Number of strains for which the diameter (mm) of the inhibition zone* was			
Type	Number of strains	6.25	6.25-13	13-18	>18
<i>Salmonella typhi</i>	212	19	15	20	158
Other salmonellae	77	5	5	10	57

* Including the width of the disk. A diameter of 6.25 mm means no inhibition.

RESULTS

In-vitro studies. The results obtained with niridazole-containing disks against various salmonella strains are shown in table I. Zone sizes less than 13 mm were scored as resistant. The percentage sensitivity was the same for both groups (74 per cent.). This level was chosen in relation to the MIC levels and the average serum levels following therapeutic doses. Tube dilution tests of the sensitive strains in table I gave MIC levels of from 4 to 8 μ g per ml. Strains with no zones of inhibition were usually sensitive to concentrations between 15 and 40 μ g per ml.

No differences were noted between disks prepared at room temperature or at 37°C. However, it was found from daily testing of disks that potency deteriorated rapidly after 4 days. After 7 days disks were almost inactive, and it is recommended that such tests should be done with freshly prepared disks. There is no advantage in using dried disks.

In-vivo tests. Mice infected with strain no. 8 were given niridazole subcutaneously 6 hr after infection and a second dose by the intraperitoneal route 3 days after infection. One group received 200 mg per kg and the other 600 mg per kg, on each occasion.

Mice infected with strain no. 12, on the other hand, were given daily

injections of niridazole by the intraperitoneal route for 5 days commencing 6 hr after infection. Two groups were treated with a dosage of 100 and 400 mg per kg respectively.

TABLE II
Effect of niridazole treatment on the 21-day mortality in mice infected with Salmonella typhimurium strains no. 8 and 12

Strain no.	Number (and percentage) of deaths within 21 days after infection in <i>S. typhimurium</i> -infected				
	control untreated mice	mice treated with niridazole (mg per kg)			
		100	200	400	600
8	19 (38)	...	5 (8.3)	...	3 (5.0)
12	25 (62.5)	14 (35.0)	...	2 (10.0)	...

Table II details the results obtained with niridazole in infected mice for strains no. 8 and 12; this shows that mice infected with strain no. 8 and treated with niridazole had a significantly higher percentage of survivors compared with the controls ($\chi^2 = 25.96$; 1 d.f.; $P < 0.001$). However, a comparison of the two treated groups did not reveal a significant difference between them ($\chi^2 = 0.252$; 1 d.f.; $P > 0.5$). Similar results were obtained for treatment of mice infected with strain no. 12 and treated with niridazole ($\chi^2 = 12.74$; 1 d.f.; $P < 0.001$), but there appeared to be some advantage in treating with 400 as against 100 mg per kg ($\chi^2 = 3.45$; 1 d.f.; $0.005 < P < 0.01$).

TABLE III
Effect of niridazole on the incidence of occurrence of S. typhimurium in the spleens of mice infected with strain no. 8

Groups of mice	Number and percentage of spleens		
	infected	sterile	total
Infected controls	31 (100)	0 (0)	31
Infected and treated with: niridazole 200 mg per kg	21 (38.2)	34 (61.8)	55
niridazole 600 mg per kg	11 (19.3)	46 (80.7)	57

where χ^2 was very close to that appropriate to $P = 0.05$. Table III gives a comparison of the proportion of infected and sterile spleens obtained from survivors infected with strain no. 8.

Two observations are relevant to the data contained in it. (1) The proportion of sterile spleens in the treated groups is significantly higher than in the

control group ($\chi^2 = 50.26$; 1 d.f.; $P < 0.001$) and (2), animals treated with 600 mg per kg had a higher proportion of sterile spleens than those treated with only 200 mg per kg ($\chi^2 = 4.05$; 1 d.f.; $P < 0.05$). By contrast with strain no. 8 no sterile spleens were found in mice infected with strain no. 12 either in control or treatment groups. However, the spleen counts of treated survivors were always lower than those of control survivors. In the control group the mean count for the 15 survivors was 7280 with a range of 5000 to 10,000 organisms. In the two treatment groups the mean counts and ranges were (a) mean 2100, range 1200–3000 and (b) mean 1840 and range 1200–5000 for the 100 mg per kg and 400 mg per kg groups respectively.

In order to test the significance of the differences of the ranked bacterial counts of the two treated groups infected with strain no. 12 the Mann Whitney U test was applied. From the data so obtained it can be concluded that the null hypothesis that a ranked bacterial count in the higher dose group is statistically equal to a count in the lower dose group can be rejected with an error of less than 1 per cent.

DISCUSSION

The efficacy of niridazole in treatment of schistosomiasis, especially due to *Schistosoma haematobium*, is well documented, though it appears to be less useful in *Sch. mansoni* and *Sch. japonicum* infections. This drug has also found a place in management of other tropical diseases, particularly amoebiasis and dracunculosis. Apart from the human studies of Neves *et al.* (1966), Kradolfer, Jarumilinta and Sackmann (1967) performed in-vivo studies with mice infected intraperitoneally with a number of Gram-negative bacteria. The ED₅₀ doses calculated for *S. typhimurium* varied from 20 to 130 mg per kg depending on the strain, though no mention is made of the inoculum size. Similarly, Hartwigk and Mulling (1967) treated calves infected with *S. typhimurium*, giving 8–10 mg per kg on alternate days for five doses; negative cultures were obtained in 16 of 23 calves so treated after completion of therapy.

The present findings confirm that niridazole is of value in prevention of death in mice exposed to *S. typhimurium* infection. Mice exposed to the less virulent strain no. 8 appeared to respond equally well to dosage regimens of either 600 or 200 mg per kg. On the other hand, mice infected with strain no. 12 responded better to a dose of 400 mg per kg than to one of 100 mg per kg. The survivors of mice infected with strain no. 8 and treated with niridazole had a significantly higher proportion of sterile spleens than control non-treated mice. In addition, there was a statistically significant increase in the number of sterile spleens in the 600 mg per kg group as compared with the 200 mg per kg group. On the other hand, treatment of infections caused by no. 12 strain did not produce sterility of the spleen in any of the survivors of either treatment group. However, the spleen counts of the treated group were lower than counts from control mice. A comparison of the ranked bacterial counts for the two treated groups of mice infected with strain no. 12 shows that spleens of mice treated with 400 mg of niridazole per kg contained significantly fewer organisms than mice treated with 100 mg per kg.

It remains to be seen whether niridazole has any part to play in the routine management of enteric fever in man. Limited experience of the treatment of two patients with typhoid fever did not support such a view. However, cases of chronic salmonellosis have not been uncommon in our experience. Most of these have been due to *S. paratyphi A* infection, but on occasion other salmonellae are involved. These present a problem in management, since they respond poorly to either chloramphenicol or ampicillin. It may be that niridazole has a place in this type of infection. It would also be of interest to determine the use of niridazole in eradication of the chronic enteric carrier state. Satisfactory control of chronic carriers is still a major problem, in spite of the apparent efficacy of ampicillin in certain cases.

SUMMARY

Groups of mice were infected with two strains of *Salmonella typhimurium*. Those infected with the less virulent strain no. 8 were treated with niridazole in doses of 200 or 600 mg per kg. Those infected with strain no. 12 received either 100 or 400 mg per kg. Compared with non-treated control mice the treated groups showed significantly higher numbers of survivors. In the group infected with strain no. 8 no difference was found in the incidence of survivors between the 200 and 600 mg per kg treatment groups. Such a difference was noted, however, in the case of strain no. 12 between the 100 and 400 mg per kg groups. Bacterial counts of spleens of survivors showed that treatment with niridazole significantly reduced the numbers of bacteria as compared with control mice.

In-vitro testing with impregnated disks was found to be effective in determining sensitivity of salmonella strains. Of 212 strains of *S. typhi* 178 gave zone sizes of 13 mm or greater. Of 77 other miscellaneous salmonella strains 67 had zone sizes of this order. It is essential that disk tests be done with freshly prepared niridazole solutions.

It is suggested that niridazole may be worthy of trial in cases of chronic salmonellosis that are unaffected by chloramphenicol and ampicillin. It may also be worth while to evaluate its use in chronic enteric carriers.

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