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Effects of the Immunosuppressive Drug Niridazole in Isogeneic and Allogeneic Mouse Tumor Systems *in Vivo*[1]

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SUMMARY

In both isogeneic (Sarcoma 1 in A/JAX mice) and allogeneic (Sarcoma 180 in C57BL/6 mice) mouse tumor systems, treatment of the tumor-bearing mice with niridazole, an antiparasitic drug, known to be a potent suppressor of cell-mediated but not humoral immunity caused enhancement of metastases to regional popliteal nodes. Niridazole also inhibited tumor growth *in vivo*, as manifested by a significant decrease in the weight of the primary tumors. The enhancement of metastases is attributed to the suppression of cell-mediated immunity by the drug, but the mechanism of tumor-growth inhibition is not yet clear.

INTRODUCTION

Niridazole, an antiparasitic drug, is the drug of choice in the treatment of schistosomiasis, a major world-wide infection of humans. No serious side-effects of this drug have been reported during its clinical use over the past 10 years in hundreds of thousands of patients. In the last 2 years, however, niridazole has been shown to suppress cell-mediated immunological responses in experimental animals (9, 10) and in man (12). More recently, it was shown that niridazole reversibly inhibits lymphokine output by sensitized lymphocytes (2) and that the drug has virtually no effect on antibody production (11).

As with any agent that suppresses cell-mediated immunity, there was concern about the extent of its effect on cellular immunity in tumor systems. We have therefore investigated the effects of this drug in isogeneic (Sarcoma 1 in A/JAX mice) and allogeneic (Sarcoma 180 in C57BL/6 mice) mouse tumor systems. The tumors were grown as foot implants on both hind feet of mice, and the incidence of metastases from the foot tumor to the regional popliteal nodes was studied. Previous studies in these systems have shown striking enhancement of metastases upon treating these tumor-bearing mice with immunosuppressive agents, such as antilymphocyte serum (7) and L-asparaginase (3, 4). In the present study, similar significant enhancement of metastases was noted in both of these tumor systems when mice were treated with niridazole. Unexpectedly, however, this drug also demonstrated inhibition of tumor growth *in vivo* as manifested by a marked decrease in primary tumor weights of the treated tumor-bearing animals.

MATERIALS AND METHODS

Mice. Male mice (C57BL/6 and A/JAX), 4 to 6 week old, were obtained from The Jackson Laboratory, Bar Harbor, Maine.

Tumors. Sarcoma 1 and Sarcoma 180 were initially obtained from The Jackson Laboratory and were maintained by serial transfers in mice.

Niridazole. This drug was obtained from Ciba-Geigy Pharmaceutical Company (Summit, N. J.). The drug was suspended in distilled water in different concentrations and 0.2 ml was administered to the mice p.o. through a blunt-tip 16-gauge metal cannula.

Implantation of Tumors and Study of Metastases. The day of tumor implant was considered Day 0 of the experimental period. For both sarcoma systems, 0.05 ml of a tumor mince, suspended 1:2 in Hanks' balanced salt solution, was injected into the s.c. tissue of the dorsum of the right and left hind feet of the mice. The mice were divided into 3 groups of 10 each. Group 1 served as the untreated controls, Group 2 was treated with rabbit anti-mouse lymphocyte globulin (0.2 ml of a 1% solution given i.p. daily from Day −3 to Day +6), and Group 3 was treated with niridazole (100 mg/kg p.o., daily from Day −3 to Day +6). The tumors were allowed to grow for 14 days, when the mice were killed and the right and left popliteal nodes were removed, treated with Zenker's fixative, sectioned, and stained with hematoxylin and eosin. On microscopy, metastatic tumor was identified as growth of sheets or clusters of tumor cells (5 or more) in the subcapsular sinus of the lymph node. The results were expressed as proportion of total lymph nodes in each group showing metastases. Also, both tumor-bearing feet were amputated from each mouse and the weight of the tumor was determined by subtracting the weight of a foot from a normal mouse from the weight of the tumor-bearing foot.

RESULTS

Table 1 summarizes the effect of niridazole on metastases in the isogeneic sarcoma-1 system in A/JAX mice, as obtained from 2 separate experiments.

It is clear from these experiments that niridazole treatment produced a significant increase in the incidence of

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Table 1

Effect of niridazole in isogeneic mouse tumor system (Sarcoma 1 in A/JAX mice)

Niridazole (100 mg/kg/mouse) was administered p.o. and ALG (0.2 ml of a 1% solution/mouse) was administered i.p., daily from Day −3 to Day +6 of the experimental period. Tumors were implanted on Day 0 and popliteal nodes were removed on Day 14.

Experimental group	Av. whole body wt (g)		Av. tumor wt (mg)	Proportion of popliteal nodes with metastasis
	Day 0	Day 14		
Group1 (23 mice): Untreated controls	22.5 ± 1.4[a]	18.7 ± 1.3	568 ± 120	10/46 (22)[b]
Group 2 (20 mice): ALG treatment from Day −3 to Day +6	24.3 ± 1.9	20.5 ± 2.5	598 ± 128	35/40 (87)
Group 3 (21 mice): niridazole treatment from Day −3 to Day +6	22.6 ± 1.8	17.9 ± 1.7	210 ± 52	27/42 (64)

[a] Mean ± S.D.
[b] Numbers in parentheses, percentages.

Table 2

Effect of niridazole in allogeneic mouse tumor system (Sarcoma 180 in C57 mice)

Experimental group	Av. whole body wt (g)		Av. tumor wt (mg)	Proportion of popliteal nodes with metastasis
	Day 0	Day 14		
Group 1 (21 mice): Untreated controls	21.9 ± 1.7[a]	18.5 ± 1.4	330 ± 24	0/42 (0)[b]
Group 2 (20 mice): ALG treatment from Day −3 to Day +6	23.8 ± 1.9	20.2 ± 2.0	380 ± 32	30/40 (75)
Group 3 (25 mice): Niridazole treatment from Day −3 to Day +6	22.0 ± 1.8	18.2 ± 1.6	210 ± 14	18/50 (36)

[a] Mean ± S.D.
[b] Numbers in parentheses, percentage.

metastases (64%) as compared with that in the untreated control mice (32%), but this effect was not as striking as that with ALG[2] treatment (87%) and that reported with L-asparaginase in our previous studies (4).

The appearance of the metastatic tumor (sarcoma 1) in the regional popliteal node is illustrated in Fig. 1. In the positive nodes, the tumor appeared as sheets of cells in the subcapsular sinus, and in some cases it had infiltrated further and occupied most of the nodal tissue.

The results also showed a significant inhibition of tumor growth in niridazole-treated animals, as indicated by decrease in tumor weights of the treated animals as compared with those in controls ($p < 0.01$). Fig. 2 illustrates the difference in the appearance of tumor-bearing feet of niridazole-treated animals compared with those in the untreated control animals. Histological examination of the primary tumors in all 3 groups showed no significant differences. Furthermore, the whole body weights of the animals in the 3 groups did not show significant differences, both at Day 0 and Day 14 of the experimental periods.

Table 2 summarizes the results obtained from 2 separate experiments in the allogeneic system, Sarcoma 180 in C57 mice. In the niridazole-treated group, there was a significant increase in the incidence of metastases (25%) as compared with that in the untreated control group (0%). However, this increase was not as striking as that noted in the ALG-treated group (75%). Our cumulative experience with this allogeneic system, now involving 1500 popliteal nodes, has shown that the incidence of metastases is extremely low (<3%) in untreated animals. The results obtained with niridazole treatment are significant at a p value of less than 0.01. In this system also, there was a significant inhibition of primary tumor growth as indicated by decrease in the tumor weights of niridazole-treated animals. The average body weights in the 3 groups, on the other hand, did not show significant differences both at Day 0 and Day 14 of the experimental periods. Histological examination of the primary tumors in all 3 groups also showed no significant differences.

DISCUSSION

Study of metastases in the isogeneic and allogeneic tumor systems, as described in this report, has proved to be particularly useful in evaluating the immunosuppressive activity of various agents. In these systems there is a definite relationship between immunosuppression of the host and the incidence of metastases of the tumor. Agents demonstrating enhancement of metastases in these systems included L-asparaginase (6), cyclophosphamide (Cytoxan), prednisone, cytosine arabinoside, and human chorionic gonadotropin (5). In pregnant female mice also a similar, significant enhancement of metastases was noted (8). The enhancement of metastases noted with niridazole in these systems was not as striking as that with ALG or L-asparaginase, the effect being comparable to that observed with agents such as prednisone, cytosine arabinoside, and human chorionic gonadotropin.

Our previous studies have shown that immunity in these

[2] The abbreviation used is: ALG, antilymphocyte globulin.

mouse tumor systems is primarily of the cellular type, as supported by (*a*) transfer by sensitized regional lymphoid cells but not by serum, (*b*) the presence of characteristic mononuclear cell infiltrate in rejecting tumor grafts, and (*c*) the *in vitro* cytotoxic effect of immune lymphocytes on the appropriate tumor cells in tissue culture (1). In preliminary experiments with the *in vitro* cytotoxic assay, we have observed a significant decrease in the cytotoxic effect of regional popliteal node lymphocytes on tissue culture-grown tumor cells in the niridazole-treated animals, compared with that in the untreated control animals. The results of these studies in the 2 tumor systems are consistent with the previous observations, that niridazole is a potent suppressor of cell-mediated immunity. In mice, this drug was shown to suppress granuloma formation around *Schistosoma mansoni* eggs and to prolong skin allograft survival across the H-2 antigens (9). Similar suppression of delayed hypersensitivity has been reported in patients with schistosomiasis receiving niridazole treatment (12). The suppression was manifested by reduction or ablation of skin reactions to various antigens and by depletion of antigen-induced lymphocyte transformation. In the animal studies referred to above (9), a single dose of niridazole, ranging from 1 mg/kg body weight to 100 mg/kg body weight, was effective; whereas in our studies a single p.o. dose of 100 mg/kg was not significantly effective, and 8 daily doses had to be administered at that level to obtain the results described.

The inhibition of tumor growth by niridazole was particularly intriguing. Since the total body weight of the animals in all the experimental groups was similar, the suppression of tumor growth could not be attributed to a systemic, cachectic effect of the drug. In previous studies with cytotoxic drugs such as cyclophosphamide, cytosine arabinoside, and L-asparaginase, we had noted similar suppression of primary tumor growth and enhancement of metastases. Since niridazole has not been reported to have any cytotoxic effect, the observed inhibition of tumor growth is difficult to explain. Thus, it appears that niridazole in high doses has a dual effect in tumor systems, first a direct inhibitory effect on the growth of the primary tumor with an immunosuppressive effect on the tumor immune response, causing enhancement of tumor metastases.

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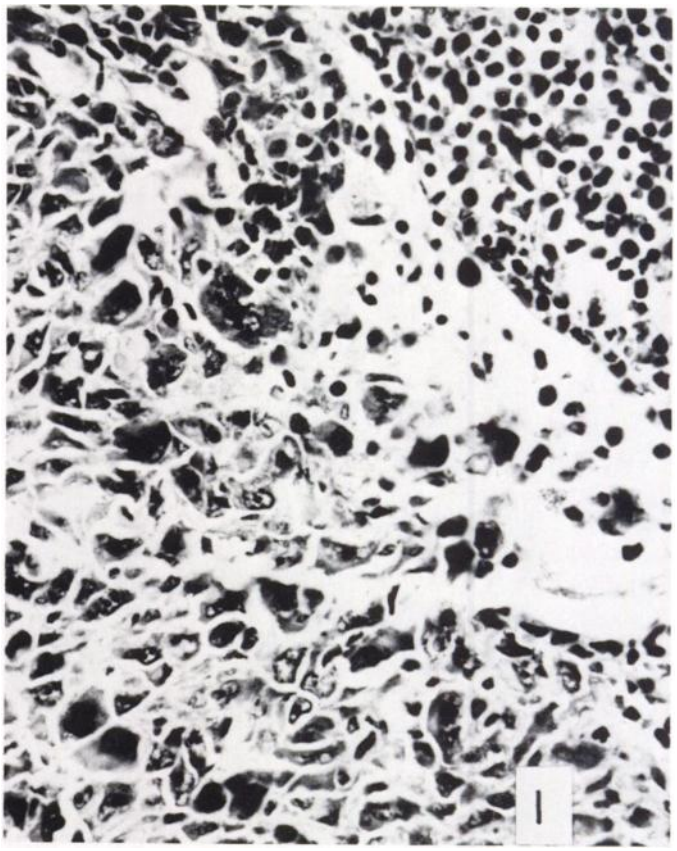

Fig. 1. Metastatic tumor in popliteal node of a mouse treated with niridazole. A/JAX mice were treated with niridazole (100 mg/kg/day) from Day −3 to Day +6 and nodes were examined on Day 14, with Day 0 being the time of tumor implant. H & E, × 400.

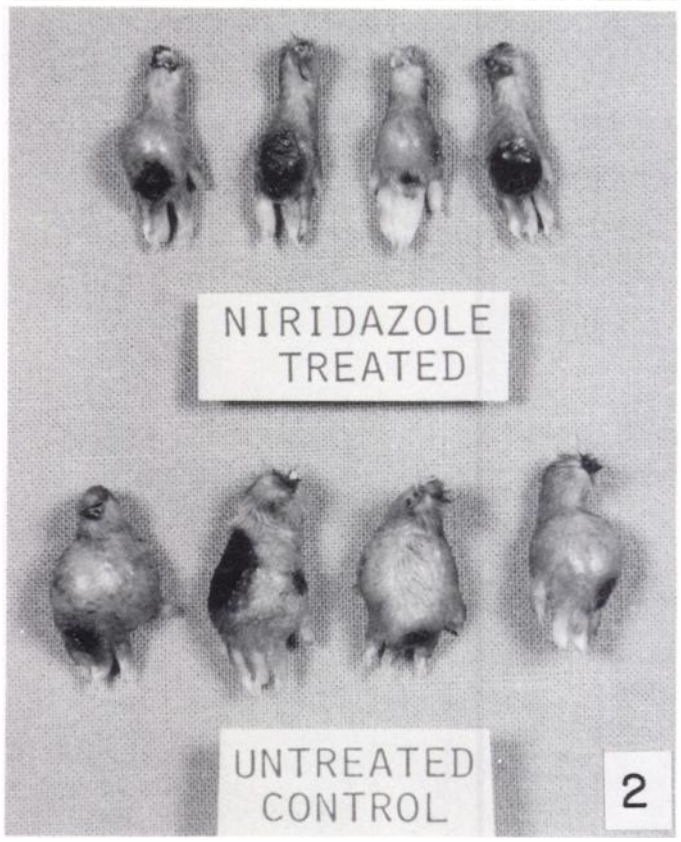

Fig. 2. Gross appearance of tumor-bearing feet from untreated control and niridazole-treated mice. Mice (A/Jax) were treated with niridazole (100 mg/kg/day) from Day −3 to Day +6, and tumor-bearing feet were amputated on Day 14, with Day 0 being the time of tumor implant. Note the smaller size of tumors in treated animals.