

Short Communication

INEFFECTIVENESS OF LEVAMISOLE AS ADJUVANT TO SURGERY WITH TWO LINES OF TRANSPLANTED RAT COLONIC CARCINOMA

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LEVAMISOLE is an agent known to be capable of restoring impaired immune response in a wide variety of clinical disorders (Symoens & Rosenthal, 1977). Some discrepancies have been seen in results of human cancer immunotherapy with levamisole (Amery & Verhaegen, 1978; Martin, 1979). Until now, neither chemo- nor immunotherapy has proved an efficient adjuvant to surgery in human colorectal cancer. We have tested the activity of levamisole on the local recurrence of tumours and the spread of metastases after surgery, in a model of transplanted intestinal cancer.

Experimental procedure

Two serially graftable tumour lines, DHB and DHD were obtained independently from adenocarcinomas induced by 1,2 dimethylhydrazine in syngeneic BDIX rats (Martin *et al.*, 1973). Levamisole was freshly prepared in phosphate-buffered saline (PBS).

Experiment 1 was designed to test the effect of levamisole on metastases. Small pieces of DHB tumour (~50 mg) were s.c. grafted in 24 rats. Tumour growth developed in 16 rats. Two months after grafting, the tumour was excised incompletely so that a recurrence might occur. Rats were randomized in two groups of 8 animals. 8 days after surgery, one group received repeated weekly i.p. injection of levamisole (2.5 mg/kg) in 1 ml PBS, the other group, weekly injections of PBS.

Recurrent tumours were excised from animals of both groups, 1, 2 or 3 times, according to the size of the tumour, to control the local tumour burden. The number of excisions was identical in both groups (2 rats once, 3 rats twice, and 3 rats thrice). The number of levamisole and placebo injections was the same for all rats (10 injections). All rats were killed 4 months after receiving the graft.

Experiment 2 tested the effect of levamisole on local and metastatic recurrence after curative excision. S.c. grafts of line DHD were transplanted into 40 rats, but only 31 rats were available for randomization and analysis. When the surface of the tumour reached ~8 cm² the tumour was removed surgically and all visible neoplastic tissue was excised. Eight days after surgery, 16 animals received i.p. 2.5 mg/kg levamisole diluted in 1 ml PBS on two consecutive days once a week and 15 animals PBS alone, until sacrifice or until the animals became moribund.

At the end of Expts 1 and 2, animals had their lungs injected post-mortem with Evans blue in order to count the number of metastases. Tumour, or graft bed, lungs, axillary, pectoral and mediastinal lymph nodes, liver, spleen, gastrointestinal tract and kidneys were systematically processed for histology.

In Expt 1, at sacrifice 6/8 rats in the levamisole group had lung metastases versus 7/8 controls. The total number of lung metastases in the levamisole group

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TABLE.—*Effect of levamisole treatment in rats with DHB or DHD intestinal adenocarcinoma transplants*

	Expt 1 (DHB)		Expt 2 (DHD)	
	Levamisole	Control	Levamisole	Control
Mean tumour weight at excision (g)	3.59 (0.68)*	4.59 (0.76)	8.53 (0.48)	8.08 (0.64)
Time between graft and excision (days)	61	61	67 (3.7)	84 (12.7)
Mean tumour weight at sacrifice (g)	4.28 (0.78)	4.10 (0.71)	9.58 (1.17)	9.88 (4.54)
Survival after excision (days)	59	59	75 (7.1)	77 (12.2)
Total survival after grafting (days)	120	120	143 (8.4)	162 (17.9)
Local recurrence	8/8	8/8	7/16	5/15
Pulmonary metastases	6/8	7/8	14/16	12/15
Mediastinal metastases	4/8	3/8	9/16	7/15
Renal metastases	0/8	2/8	6/16	7/15
Cured animals	0/8	0/8	2/16	3/15

* S.e. in parentheses.

was 44 *versus* 69 in control rats (not significant). Mediastinal spread was seen in both groups. Kidney metastases occurred only in 2 control animals. No statistically significant difference was found between control and treated animals.

In Expt 2 lung metastases were so numerous in both groups (often more than 100 in one rat) and the size of the metastatic nodules varied so much (from 0.1 mm to 1 cm in diameter) that it was impossible to count and evaluate them quantitatively. There was no significant difference between levamisole-treated rats and controls, either in survival after surgery or in local recurrence and metastases. The number of cured animals was rather low, 2 out of 16 in levamisole group, 3 out of 15 in control group. Results of Expts 1 and 2 are summarized in the Table.

Since the report of Renoux & Renoux (1972) who obtained complete prevention of recurrence and a reduction in pulmonary metastases in mice grafted with Lewis tumours, contradictory results have been obtained showing no effect (Hard, 1977; Hopper *et al.*, 1975; Potter *et al.*, 1974), some decrease in tumour growth and dissemination (Aleksic *et al.*, 1977) or an enhancing effect (Fidler & Spitler, 1975; Sampson *et al.*, 1977). Amery *et al.* (1977) define the best conditions for drug efficiency: (1) The best results are obtained with 2.5 mg/kg. (2) Levamisole is more effective on slow-growing tumours. (3) It affects preferentially metastasis forma-

tion. (4) It should be used as an adjuvant treatment.

In this work levamisole has been tested as an adjuvant to surgery, at the dosage of 2.5 mg/kg. Colonic carcinoma transplants are growing slowly; the mean tumour take is 20 days for DHD and 25 days for DHB (Martin *et al.*, 1976). In Expt 1, only the spread of metastases was studied, since local recurrence had purposely been allowed to occur by incomplete tumour excision. In Expt 2, total tumour resection was performed, but the probability of recurrence or metastasis was high, since tumour weight was important at the time of surgery. The situation was very close to that of patients with human Dukes' B or C colonic carcinoma. Even when the conditions of levamisole administration were very close to the "ideal" situation defined by Amery *et al.* (1977) our results were entirely negative. The extrapolation of results obtained in experimental work to the clinical situation is difficult; however the data reported above do not support the treatment of human colorectal cancer with levamisole.

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