Immunopotentiation with Levamisole in Resectable Bronchogenic Carcinoma: A Double-blind Controlled Trial

Study Group for Bronchogenic Carcinoma

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Summary

A long-term multicentre double-blind study was designed to test the immunomodulating effects of levamisole in patients undergoing operation for primary bronchial carcinoma. They received levamisole 50 mg three times a day by mouth or placebo for three days every fortnight, starting three days before surgery. Unless there was clinical evidence of recurrence, cytostatic drugs, corticosteroids, and radiotherapy were prohibited.

In the 111 patients who have been followed up for one year the incidence of side effects was similar in both groups. Recurrences occurred in 10 out of 51 patients (seven deaths) receiving levamisole and in 20 out of 60 (12 deaths) receiving placebo. Further analysis showed that there were fewer recurrences on levamisole in patients with squamous cell carcinomas and medium and large primary tumours and fewer suspected and proved recurrences and deaths from metastases on levamisole in patients with extended tumours. Distant recurrences tended to be less common with levamisole, whereas the disease-free interval in relapsing patients was almost identical in the two groups. These interim results show that levamisole seems to exert its beneficial effect by preventing immunosuppression due to surgery.

Introduction

Tumour immunology arouses much interest, and several investigators are particularly concerned with lung cancer. Yet data on the antigenicity of bronchogenic carcinoma are scarce and equivocal, but there are good reasons for accepting that squamous cell carcinoma is immunogenic. On the other hand, most investigators have described a defective cellular immunity in patients with lung cancer, especially in non-resectable cases; rough assessments of cellular immunity, such as skin tests with unrelated antigens, in operable cases yield almost normal or only slightly depressed results, but more sophisticated tests detect some T-lymphocyte abnormalities even in patients with very small tumours. Also, most investigators have found a correlation between operability or survival time, or both, and T-cell reactivity.

Levamisole is an anthelmintic, but a few years ago it was found to possess immunopotentiating properties; it seems to restore inefficient host defence mechanisms. In most animal cancer models levamisole does not influence the growth of the primary tumour but prolongs the remission period after chemotherapy. In patients with cancer levamisole restores skin reactivity to 2,4-dinitrochlorobenzene (DNCB) and purified protein derivative (P.P.D.).

In view of the immunomodulating effects of levamisole and the expected benefit of immunopotentiation in cancer patients with minimal tumour residue we tested this drug in patients undergoing surgery for resectable bronchogenic carcinomas. We report here the results obtained in the first 111 patients, who have been followed up for at least one year.

Patients

Altogether 111 patients who had undergone operation for primary bronchial carcinoma a year earlier were considered for this study. There were 103 men and five women (in three patients the sex was not recorded) aged 40 to 80 years (median 60 years). Their weights before operation ranged from 53 to 121 kg (median 74 kg). Before entering the trial the erythrocyte sedimentation rate (E.S.R., first hour) was 2 to 115 (median 23) mm. Seventy-one patients had squamous cell carcinoma, 21 had adenocarcinoma, 17 had other types of bronchial carcinoma, and in two patients no histological diagnosis was available. Tumours of the right and left lung were equally common and just over half the tumours were peripheral. Lobectomy was performed in 68 patients, pneumonectomy in 33, and a sleeve operation in one (no data available in nine patients). During the last weeks before surgery none of the patients had regularly smoked 0 to 70 cigarettes a day (median 20).

Methods

Levamisole 50 mg three times a day by mouth or placebo had been given for three days before surgery, and this three-day course of treatment was repeated every fortnight thereafter. The medication was individually coded and strictly double-blind. Patients were randomly assigned to placebo or levamisole, and the randomization was performed separately for each centre. Patients, who in spite of thorough exploration before, proved inoperable at surgery or showed no malignancy at post-surgical pathological examination were excluded from the trial.

Double-blind treatment and follow-up examinations will be continued in these patients until they develop clinical evidence of recurrence or for at least two years after surgery. The interim analysis reported here was performed after 111 patients had been followed-up for at least one year. The whole study will be completed when 200 patients have been followed for two years.

At selection (at least 15 days before the operation) data on the patient, his tumour location, and his smoking habits were recorded, and a sensitization test with 2 mg DNCB was performed. Five days before surgery a test with 25, 50, and 100 μg DNCB and an intradermal reaction with 10 IU of P.P.D. were performed, followed by an assessment 48 hours later—that is, just before the start of treatment. These skin tests were repeated 10 days and two months after surgery. After surgical removal the tumours were fixed with formalin 10% Boun fixative and their greatest and smallest diameters were measured.

To establish the regional extent of the tumour a slightly modified category grouping system, previously described by Slack, was adopted: category 1 included those lesions limited to the lung with no positive lymph nodes and either with (a) or without (b) evidence of blood vessel invasion; category 2 included resectable lesions confined to the lung and hilar or mediastinal lymph nodes; category 3 included
cases in which there was evidence that not all the tissue involved had been resected at operation either because there was (a) histological evidence of tumour in the resection margin or on the thoracic wall (including parietal pleura) or because (b) the surgeon considered that tumour tissue had not been removed completely.

We combined the indices on size and extent of the cancer into two gradings: grade I included all primary lesions with a largest diameter <3 cm, except those with a positive resection margin (category 3), and all lesions of category 1b, except those with a largest diameter >7 cm; grade II included all other combinations of categories and tumour sizes.

After discharge from hospital all patients attended for examination every second month and when recurrence was suspected. If recurrence was proved the location was registered and the patient treated appropriately. Unless there was clinical evidence of recurrence cytostatic drugs, corticosteroids, and radiotherapy were prohibited throughout the study.

All data were stored in an I.B.M. 370/135 computer and, unless otherwise stated, analysed by means of the two-tailed Fisher exact probability test using a Wang 2200 computer.

Results

On breaking the code we found that the two treatment groups were comparable in length of follow-up, general description (age, sex, weight, and smoking habits), skin test reactivity, and type, location, and extent of the tumour (median test or χ² test, two-tailed probability for both).

The incidence of side effects was roughly similar in the two groups, as previously reported, but there was slightly more gastric intolerance, nervousness, and slight fever in the levamisole group.

Recurrence was suspected and proved in 10 of the 51 patients on levamisole, and death from carcinoma was registered in seven of them. Recurrence was suspected in 23 of the 60 patients on placebo; 20 patients proved to have recurrences and 12 of these died from cancer. Hence, there was a clear but not statistically significant trend in favour of levamisole, and this was true for all three co-operating centres. Twelve patients on levamisole died from causes other than cancer (three as a result of surgery, three from causes unrelated to the disease, and six from unknown causes), as did 14 on placebo (three as a result of surgery, one from causes unrelated to the disease, and 10 from unknown causes).

Further analysis showed that the difference between levamisole and placebo in the number of proved recurrences was related neither to most of the general data on patients, except for the habit of smoking, nor to skin test reactivity (table I). Among patients who smoked more than 10 cigarettes daily recurrence was suspected in 21.2% of those on levamisole and 47.5% of those on placebo (P=0.027), and the respective percentages for disease mortality were 18.2% and 27.5% (P>0.05).

Levamisole activity was, however, more dependent on characteris-
were also fewer deaths from carcinoma among the levamisole-treated group (15·0% v. 33·0%), though the difference was not significant.

The above data were also confirmed by the findings concerning the total extent gradings. There was no difference between levamisole and placebo among grade I patients, but among grade II patients 20·0% of those on levamisole had suspected recurrences compared with 73·9% of those on placebo (P<0·002). On levamisole 6·7% died from cancer compared with 43·5% on placebo (P<0·026).

Since we expected that several of the indices which were relevant in assessing the effectiveness of levamisole could be interrelated we analysed their relationships (table III). As expected, the size and the regional extent category were interrelated, but patients who smoked more than 10 cigarettes daily had larger and more extensive tumours than the other patients. On the other hand, patients with squamous cell carcinoma tended to have a tumour extent similar to that of the other patients.

The incidence of intrathoracic recurrences was similar in the two groups (six (11·8%) in levamisole group, seven (11·7%) in placebo group), but distant recurrences (in bone, brain, and liver) tended to be less common on levamisole than on placebo (four (7·8%) and 13 (21·7%) respectively; P<0·06).

The disease-free interval—that is, the time between surgery and evidence of recurrence—in patients who relapsed is shown in fig. 2.

Discussion

This first interim analysis has shown some promising results on levamisole: recurrences in patients treated with levamisole were about half as common as those in patients on placebo. That this favourable trend was found in all three centres suggests that the difference was not accidental.

Intrathoracic recurrences were not diminished by levamisole treatment, though it is hard to say why. If it was not purely accidental, postsurgical changes (such as fibrosis and scar formation) may have made the intrathoracic area an immunologically privileged site. Levamisole treatment had an apparent influence on distant metastases, possibly because it prevented the haemotogenous spread of the tumour during surgery. It is well known that during operation cancer cells appear in the blood, but most of these cells fail to produce metastases.

One of the factors furthering metastatic implantation and growth of such cells is the immunosuppressive effect of surgery, which affects the tumour-specific cell-mediated immunity. Since levamisole was started three days before surgery such immunosuppression might have been prevented in patients who received the drug. Further research may provide an answer to the question.

Quite unexpectedly the skin tests were found to be irrelevant in predicting whether levamisole would have a good effect. It should be borne in mind, however, that skin reactivity was almost normal in our group of patients. The most important factors in predicting benefit from levamisole treatment were the histological diagnosis and the extent of the tumour. There are good reasons for accepting squamous cell carcinoma as immunogenic, and thus it is not surprising that it should be affected beneficially by immunopotentiation. The explanation of the importance of the extent of the tumour (which also seemed to be responsible for the good results on levamisole in heavy smokers) might be found in animal experiments, which have shown that tumour-specific immunity is depressed in animals with large tumours, that this depression is greater when the tumour is larger, and that immunity is restored after cytoreductive therapy—that is, surgery or radiotherapy—but after a certain latent period. The latent period for the reappearance of specific cell-mediated immunity is also related to the type of experimental tumour model. Hence, levamisole seems to exert its beneficial effect, at least in part, by preventing immunosuppression due to surgery, especially in patients with large tumours and, therefore, with pre-existing suppression of tumour-specific immunity.

For future research with levamisole patients should be classified according to the extent of their tumour, levamisole should be started before surgery, and the site of recurrence should be registered carefully.

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References

Carrier Solutions for Low-level Intravenous Insulin Infusion

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Summary

In the use of low-level intravenous insulin infusion for treating diabetic hyperglycaemia and ketosis, infusion apparatus results in significant losses of 60-80% of insulin in dilute physiological saline solution (40 U/l). It is therefore necessary to add protein to the carrier solution to minimize losses and maintain a constant delivery rate. Recovery studies showed that 3.5%, w/v polygeline solution (polymer of degraded gelatin) was a suitable medium for this purpose, offering some advantages over human serum albumin. A minimum concentration of 0.5% polygeline was required to ensure adequate delivery of insulin to the patient.

Introduction

Recent reports have shown the effectiveness of low-dose intravenous insulin infusion in the treatment of diabetic hyperglycaemia and ketosis. The observations that insulin is adsorbed to glass and plastic surfaces, however, emphasize that caution is necessary to ensure that sufficient insulin reaches the patient to be effective. It has been reported that 55% of added insulin is adsorbed by glass and polyvinyl chloride containers within 15 seconds of addition of insulin to Ringers' lactate solution, and that a typical intravenous infusion set adsorbs another 50% of the remaining insulin.

Reports have shown that addition of human serum albumin (H.S.A.) to dilute solutions containing insulin greatly reduces insulin adsorption. We have used gelatin solutions to mimic the action of serum in insulin radioimmunoassay, and in later assays substitution of a commercial 3.5% polygeline solution (polymer of degraded gelatin; Haemaccel, Hoechst- Behringwerke) proved equally effective. It seemed likely that this medium, which is normally used as a plasma substitute, would also be suitable as an insulin carrier solution.

We describe here studies of the effectiveness of polygeline as a carrier solution for low-level insulin infusion for periods of up to eight hours.

Methods

We added 20 units of crystalline porcine insulin (Novo Actrapid, concentration 40 kU/l) to the following standard 500-ml containers: (a) a plastic container holding polygeline 3.5%; (b) a glass container holding polygeline 3.5%; (c) a glass container holding saline 0.9% and H.S.A. 2% (Commonwealth Serum Laboratories, Melbourne); (d) a glass container holding saline 0.9%; and (e) a plastic container holding saline 0.9%.

The solutions were mixed by agitating the containers, and 15 minutes after the addition of insulin aliquots were taken for assay. The containers were then connected to controlled paediatric drip sets and each adjusted to a flow rate of about 1 ml/min. The effluent was collected for insulin assay after two and a half, five, and eight hours. The experiment was performed at 22°C. Immediately after collection samples were frozen in plastic tubes and stored at −20°C. Later they were thawed, diluted to 1/700, and assayed for immunoreactive insulin using a commercial radioimmunoassay kit (Soris, Italy). The quoted content of the original insulin phial was also checked by radioimmunoassay. In pilot studies insulin standard curves set up in buffer and in the above carrier solutions diluted to 1/700 were equivalent.

In a further experiment to investigate the relation of insulin recovery to concentration of polygeline in the carrier solution 500-ml glass infusion containers were set up with saline as a diluent to produce a range of concentrations of polygeline from 0 to 3.5%. After addition of insulin as in the first experiment the solutions were left standing at 22°C