Dose Dependence of Immunopotentiation and Tumor Regression Induced by Levamisole

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SUMMARY

Breast cancer was induced in female Sprague-Dawley rats by 7,12-dimethylbenz(a)anthracene. Once tumors had become established, they were treated with varying doses of the immunopotentiating drug, levamisole. Tumor growth was measured in the various dosage groups, and at 6 months after tumor induction the animals were sacrificed. Their immunological competence at this time was measured by the mitogen responses of splenic lymphocytes.

Untreated animals with breast cancer were found to be immunosuppressed compared to normal animals. The drug levamisole resulted in immunopotentiation, but at high doses it was immunosuppressive. Tumor regression was observed at doses that resulted in immunopotentiation, but not at high doses. There was a significant correlation between immune competence and tumor regression. It is concluded that levamisole can cause regression of breast cancer in the rat but that this effect is critically dependent on the dose of the drug; these observations confirm previous studies carried out on human cells in vitro. It is recommended that high doses of the drug be avoided in human clinical trials and that the patients who receive this drug should have their immune responses carefully monitored.

INTRODUCTION

The anthelmintic drug, levamisole, has been shown to potentiate cellular immunity (5, 7, 8, 10, 14); since it is a relatively nontoxic thiazole derivative (Chart 1), this property has led to clinical trials in human breast cancer (11) and in other cancers (1, 14, 15). However, in animal tumor systems the efficacy of the drug has been variable. Thus, the dose of the drug; these observations confirm previous studies carried out on human cells in vitro. It is recommended that high doses of the drug be avoided in human clinical trials and that the patients who receive this drug should have their immune responses carefully monitored.
and, in fact, tended to have larger tumors than did the controls (p < 0.005). However, those animals that received the tumors were consistently smaller than those seen in the controls, although this was not statistically significant. The decrease in size of the tumors in animals on the high dose of levamisole that was observed toward the end of the study was simply a reflection of the fact that many of these animals died of their cancer at this time, these being the ones with the largest tumors.

Table 1 is a summary of the state of the animals at the end of a 6-month period from tumor induction. Animals that did not receive DMBA did not develop tumors, whereas two-thirds of the animals given this carcinogen developed breast cancer. There was no evidence of tumor regression in untreated animals whereas, in contrast, many animals given levamisole, 2 or 4 mg/kg, showed evidence of tumor regression as measured by disappearance or decrease in size. However, there was no instance of tumor regression in those animals treated with high doses of levamisole at 8 mg/kg. Deaths in the control group and among those animals that did not develop tumors were due to pneumonia.

The relationship between mean tumor diameter at the end of the study and dose of levamisole is shown in Chart 3, and again it shows that the optimum dose of levamisole is 2 to 4 mg/kg. Doses in excess of this do not inhibit tumor growth. The effect of different doses of levamisole on cellular immunity as measured by responses of splenic lymphocytes to vegetable mitogens is shown in Table 2. The values given are cpm/culture and are the mean of triplicate observations in each experiment. The mitotic indices calculated from the data shown in Table 2 for the 3 mitogens PHA, Con A, and PWM were plotted against the dose of levamisole that the animals received (Chart 4). Chart 4 shows that untreated animals with breast cancer have slightly impaired responses compared with normal animals, whereas those animals that received doses of levamisole between 2 and 4 mg/kg show evidence of immunopotentiation. This was most evident when Con A was used as the mitogen, suggesting activity on a T-cell population.
The effect of levamisole on state of animals with breast cancer at end of 6-month observation period

<table>
<thead>
<tr>
<th>Animal</th>
<th>Total No.</th>
<th>No. alive</th>
<th>No. with tumor</th>
<th>No. with regression</th>
<th>No. dead with tumor</th>
<th>No. dead without tumor</th>
<th>Size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal animal</td>
<td>30</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0*</td>
</tr>
<tr>
<td>Untreated breast cancer</td>
<td>30</td>
<td>10</td>
<td>9</td>
<td>0</td>
<td>11</td>
<td>9</td>
<td>8.5</td>
</tr>
<tr>
<td>Levamisole, 2 mg/kg</td>
<td>30</td>
<td>21</td>
<td>10</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>Levamisole, 4 mg/kg</td>
<td>30</td>
<td>23</td>
<td>10</td>
<td>9</td>
<td>2</td>
<td>5</td>
<td>1.7</td>
</tr>
<tr>
<td>Levamisole, 8 mg/kg</td>
<td>30</td>
<td>23</td>
<td>23</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>6.9</td>
</tr>
</tbody>
</table>

* Mean diameter of all tumors in the respective groups.

The relationship between mitotic index for the 3 mitogens and tumor diameter is shown in Chart 5. There was an excellent linear negative correlation between immune competence and tumor size for all mitogens (for Con A, \( r = -0.98, p < 0.001 \); for PHA, \( r = -0.88, p < 0.05 \); and for PWM, \( r = -0.94, p < 0.01 \)).

**DISCUSSION**

This study was stimulated by the realization that the immunopotentiating effect of levamisole was critically dose dependent and that high doses of the drug could be immunosuppressive. This was the result of a previous investigation (13) in which we showed that levamisole could potentiate a mixed-lymphocyte culture or the response of human peripheral blood lymphocytes to mitogens in vitro but that, in this system, immunosuppression occurred at high doses. The present study confirms the importance of the dose of levamisole. In a dose range of 2 to 4 mg/kg, there was clear evidence of inhibition of tumor growth and even evidence of

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[Charts and tables as described in the text are not included in this response.]
tumor regression; but at higher doses this was not observed and, in fact, the spread of cancer tended to be worse than that in untreated animals. This correlated very well with the immune competence of the animals in a negative way; i.e., those animals showing the greatest immunopotentiation had the least tumor mass. In this study, tumor mass was measured in terms of mean tumor diameter, but when this is converted to volume the effects are even more marked since tumor mass variation would be raised 3 logs.

These observations do not prove that tumor inhibition was a direct result of immunopotentiation induced by levamisole, but this is the most likely explanation of the results. It could be argued that the drug itself has antitumor activity, but this is unlikely for 2 reasons: (a) direct antitumor activity of levamisole has not been reported; and (b) the immunopotentiation seen to accompany decrease in tumor size is unlikely to be due to direct removal of tumor since the immune competence of the optimum treatment groups was well above normal.

Our observations explain, in part, some of the controversy surrounding the use of levamisole in animal tumor systems. Several of the reports indicating a lack of effect of the drug (4, 6) were based on studies in which high doses of the drug were used, and it is likely that these doses were immunosuppressive. On the other hand, those studies that demonstrate antitumor activity used lower doses of levamisole (3, 12).

In addition, varying routes of administration of the drug have been used, and there are almost certainly variations in the antigenicity of the tumor systems studied. The critical nature of the dose of levamisole may represent a more general biological phenomenon that has considerable importance in attempts to manipulate immune responses. It has been observed that many dose-response relationships that modify the activity of the reticuloendothelial system are not linear but frequently result in the production of biphasic M- or W-shaped curves (2). Our observations may be a specific instance of such a phenomenon that reflects activity on separate cell populations or interference with negative feedback control systems.

The optimum dose of levamisole indicated by this study appears to be 2 to 4 mg/kg, and this agrees closely with the recommended human dose of approximately 150 mg/day. The dose-response curves for mitotic activity in the rat bear a striking resemblance to those that we previously reported (13) in the in vitro human studies.

In view of the increasing development of clinical trials of levamisole in human cancer and the resultant encouraging results thus far obtained (11), we believe that it is of great importance that recommended dose levels of the drug not be exceeded for fear of inducing an immunosuppressive state. Ideally, all such clinical trials should include some monitor of immune competence in the patient in order to demonstrate that the objective of immunopotentiation is, in fact, being achieved.

REFERENCES

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