Enhancement of Mammary Tumor Formation in Mice by a Cytostatic Drug, Melphalan

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SUMMARY

Melphalan (L-phenylalanine mustard), a cytostatic drug used in treatment of human breast cancer, was injected into mice bearing preneoplastic hyperplastic alveolar nodule mammary outgrowth line D2. Melphalan, in doses of either 2.5 or 5.0 mg/kg, markedly enhanced tumor formation in its mammary nodule outgrowth line. Samples of nodule outgrowths in untreated mice produced 13% tumors by 9 months after transplantation, whereas nodule outgrowths in melphalan-treated mice produced 57% and 85% mammary tumors in the lower and higher dose groups, respectively.

INTRODUCTION

Since the original discovery by Haddow et al. (6) that cytostatic drugs may be carcinogenic, evidence has accumulated in both experimental animals (15) and humans (14) indicating that a number of cytostatic drugs may exhibit carcinogenic potential. In a recent study by Weisburger et al. (15), 13 different cytostatic drugs were tested for their carcinogenic potential in mice and rats. Both alkylating and nonalkylating drugs produced a variety of tumors in both mice, a strain free of expressible mammary tumor virus. Other reports (2, 8) have shown that adriamycin and daunomycin, a strain free of expressible mammary tumor virus. Other reports (2, 7) have shown that adriamycin and daunomycin enhance mammary tumor formation in rats, but thus far little data have been reported for mice.

The question of whether the carcinogenicity of these drugs is a frequent or a very remote event in humans undergoing chemotherapy for a variety of neoplastic and non-neoplastic diseases is still debatable (13). However, the question assumes more importance as evidence has increased in the experimental literature. In the experiments presented here, BALB/c mice bearing a preneoplastic HAN population were treated with a cytostatic regimen of melphalan (9) to determine the inhibitory effects of this drug.

MATERIALS AND METHODS

All mice were BALB/c female mice, bred and maintained in the closed mouse colony in the Department of Cell Biology at Baylor College of Medicine. Mice were housed 5 to 6 to a cage and maintained in temperature- and light cycle-controlled rooms. The mice were fed Wayne Lab Blox and water ad libitum. BALB/c mice are free of expressible mammary tumor virus (3).

Melphalan was prepared fresh, dissolved in distilled water, and injected i.p. immediately after preparation. Melphalan was injected in concentrations of either 2.5, 5.0, or 10.0 mg/kg body weight. The mice were given injections once a week for 6 consecutive weeks starting when the mice were 6 weeks old. All mice were weighed weekly between 6 and 12 weeks of age and then every 3 weeks thereafter.

Samples of nodule outgrowth line D2 were transplanted into the mammary gland free fat pads of 3-week-old syngeneic mice, according to the method of DeOme et al. (4). The tissue grows and fills the fat pad as alveolar hyperplasia within 8 to 10 weeks (12). In Experiment 1, 9 weeks after transplantation, the fat pads containing the transplants were processed for whole mounts as previously described (10). The percentage of fat pad filled by the nodule outgrowths was calculated by examining the stained whole mounts under a dissecting microscope fitted with a grid in the ocular and determining the area occupied by the nodule tissue. In Experiment 2, control and treated mice were left to develop mammary tumors and were palpated weekly. Mammary tumors were fixed in formaldehyde solution, sectioned at 5 μm, and confirmed histologically.

Nodule line D2 is a well-characterized line of preneoplastic HAN outgrowth that arose from a primary HAN found in an 18-month-old BALB/c female mouse (12). It has been serially transplanted in the mammary gland free fat pads of 3-week-old syngeneic mice since 1965 and is now in its 38th transplant generation.

RESULTS

The effect of melphalan on the growth of mammary nodule line D2 in the mammary fat pads is shown in Table 1. Mice exposed to melphalan exhibited a slight decrease of weight at the end of the drug treatment (12 weeks old). This was significant only for mice given the high dose. Only the transplants exposed to the highest dose (10 mg/kg) showed any inhibition of growth. Of 12 transplants, 5 (42%) showed a growth response that was less than the minimum response seen in control mice. The mean percentage of inhibition of growth of the transplants in the treated group was 30%.

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The effect of melphalan on the tumor potential of nodule line D2 is shown in Table 2. Mice exposed to melphalan, 2.5 and 5.0 mg/kg, produced 57 and 85% mammary tumors, respectively, compared to 13% for control mice. Mice exposed to 10 mg/kg died by 17 weeks due to cytotoxic effects of the drug. The time for 50% of the transplants to produce mammary tumors was 30 weeks for the low dose (2.5 mg/kg) and 21 weeks for the medium dose (5.0 mg/kg). All mice were of similar body weights by 21 weeks of age.

DISCUSSION

The results reported here indicate that melphalan, in a therapeutic dose regimen, produced a high incidence of mammary tumors. Contrary to the initial expectations when the experiment was started, melphalan was demonstrated to be a potent mammary carcinogen in this murine model system, producing 57 and 85% mammary tumors at doses of 2.5 and 5.0 mg/kg, respectively. The total dose of melphalan given in the 2 groups of mice was approximately 0.25 and 0.5 mg/mouse, respectively. The carcinogenicity of melphalan was equal to if not more potent than that of 7,12-dimethylbenzanthracene, a well-documented mammary carcinogen. In this system, 1.5 mg of 7,12-dimethylbenzanthracene per mouse result in a similar incidence with a similar latent period (12). The carcinogenicity of melphalan was not correlated with its cytostatic effect, since the 2 lower doses (2.5 and 5.0 mg/kg) had no measurable cytostatic effect; whereas the high dose (10 mg/kg) inhibited weight gain and growth of nodule tissue and eventually led to death of the mice by 6 weeks after the last dose of the drug.

These results are important since they demonstrate the potential carcinogenicity of melphalan. In other experiments, Weisburger (15) found no effect of melphalan on mammary tumorogenesis in the Sprague-Dawley rat and the Swiss-Webster mouse, although tumors developed at other sites. It may be that melphalan, like 7,12-dimethylbenzanthracene, is very effective in enhancing the nodule to tumor transformation (12) but is weakly effective in altering normal cells to produce HAN and subsequently tumors (11).

Human breast cancer is a disease affecting both breasts (5, 16), and individuals with prior breast cancer are high-risk patients for subsequent breast cancer in the contralateral breast (5, 18). In addition, the disease is suspected of passing through preneoplastic states (1, 7, 16). For these reasons, the indication of a high carcinogenic potential for melphalan under certain conditions warrants careful follow up of patients who have received melphalan, to detect possible adverse secondary effects.

ACKNOWLEDGMENTS

I wish to acknowledge the expert guidance of Dr. Florence White, of the National Cancer Institute, in the use of cytostatic drugs and for the generous supply of melphalan. I also wish to acknowledge the expert technical assistance of Frances Shepherd and Tim Gropp.

REFERENCES

14. Sieber, S. M., and Adamson, R. H. Toxicity of Antineoplastic Agents in

Table 2

Effect of melphalan on tumor potential of nodule line D2

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>12 wk</th>
<th>21 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>22.7 (15)</td>
<td>24.9 (15)</td>
</tr>
<tr>
<td>5.0</td>
<td>22.5 (14)</td>
<td>24.9 (7)</td>
</tr>
<tr>
<td>10.0</td>
<td>18.2 (12)</td>
<td>Dead by 17 wk</td>
</tr>
</tbody>
</table>

# TE<sub>50</sub>, time for 50% of the transplants to produce tumors; tumor incidence calculated from time after transplantation.

* Numbers in parentheses, number of transplants.

Table 1

Effect of melphalan on in vivo growth of mammary nodule line D2 in the mammary fat pad

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>No.</th>
<th>Wt (g)</th>
<th>% fat pad filled</th>
<th>% inhibition of growth</th>
<th>% showing inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>5</td>
<td>22.8 ± 0.6</td>
<td>93 (9)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.0</td>
<td>6</td>
<td>23.7 ± 0.7</td>
<td>82 (11)</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>10.0</td>
<td>6</td>
<td>21.9 ± 0.5</td>
<td>66 (12)</td>
<td>30</td>
<td>42</td>
</tr>
</tbody>
</table>

# Numbers in parentheses, number of transplants.

* TE<sub>50</sub>, time for 50% of the transplants to produce tumors; tumor incidence calculated from time after transplantation.

* Numbers in parentheses, number of transplants.

* Controls have been transplanted for 9 months.

* Rest of mice in this group had large tumors, thus they were not used for the weight calculation at this time period.


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