Effect of Orotic Acid on the Metastasis of Mammary Tumors in Mice

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SUMMARY

In the course of studying the effect of nucleic acid precursors on viral carcinogenesis it was observed that an increased number of metastases developed in the lungs of orotic acid-treated mice with spontaneous mammary tumors. Further evidence that orotic acid increases the establishment of lung metastases was noted when orotic acid-treated C3H/HeN mice given injections intravenously of a cell suspension of mammary tumor cells were found to have significantly more lung metastases than control mice which received only the intravenous tumor cell injection.

Pentosenucleotides (1) DNA hydrolysate and the components and precursors of nucleic acids, particularly the pyrimidine series (2), have been shown to reduce the number of pulmonary tumors induced in mice by urethan. An inhibiting effect by the pyrimidine precursors on methylcholanthrene induction of pulmonary adenomas in mice has also been demonstrated (3). These reports of the inhibition of chemical carcinogenesis by pyrimidine precursors suggested that a study of the effect of a pyrimidine on viral carcinogenesis would be interesting.

Consequently, Part I of this report was designed to compare the incidence of milk agent-induced mammary tumors in mice receiving the pyrimidine, orotic acid, with the tumor incidence in untreated animals. The second part of this report evolved from observations made in Part I. Because examination of the organs and tissues from tumor-bearing orotic acid-treated mice revealed an increase in the number of mammary tumor metastases to the lungs, additional experiments were undertaken to study this apparent enhancement of the metastatic process by orotic acid. Part II is a report of these findings.

MATERIALS AND METHODS

Part I.—Female C3H/HeN mice which had ingested the mammary tumor milk agent during the nursing period were used in this study. At 5 months of age these mice were divided into two groups. One group (34 mice) received 0.1 per cent orotic acid1 added to the drinking water, and the other group (37 mice) received tap water alone. All were fed Purina Laboratory Chow pellets. The animals were examined once a week, and the time of development of mammary tumors was noted. Any mice in which tumors with a mean diameter of 2 cm. could be palpated were killed, and all survivors were killed when 21 months of age.

Representative sections were taken from the organs and tissues. Special attention was given to the lungs, and coronal sections were made at three levels through each lung. The tissues were fixed in 10 per cent neutral formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Part II.—Forty-nine female C3H/HeN mice, 3 months old, divided into four groups of twelve, twelve, sixteen, and nine animals per group, were given injections subcutaneously in the interscapular region of 1 ml. of 0.5 per cent orotic acid in distilled water daily for 5 days. The dose of orotic acid was then reduced to 0.5 ml. daily and was given five times per week throughout the entire experimental course.

After the initial five injections of orotic acid, the animals received an intravenous injection into the tail vein of 0.25 ml. of a tumor cell suspension prepared in the following manner. Spontaneous mammary tumors obtained from C3H/HeN mice were passed through a tissue press with 2-mm. holes in the screen. Approximately 25 mg. of this tumor mash was suspended in 6 ml. of 0.85 per cent saline. The suspended tumor fragments were then passed through a series of hypodermic needles of decreasing size from 18- to 26-gauge. Cell counts of the tumor cell suspension in a hemocytometer revealed approximately 1.5 million cells/ml. Thus a separate tumor was used to inject each group of experimental (orotic acid-treated) and its respective control animals. Care was also taken to keep the suspension well mixed during the time the animals were given injections.

For controls, 68 mice of the same strain and age were used. They were divided into eighteen, eighteen, twenty-one, and eleven animals per group. Each control group received the same amount of intravenous tumor suspension at the same time as its respective experimental groups.

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1 Purchased from Nutritional Biochemical Corporation, Cleveland, Ohio.

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EFFECT OF OHOTIC ACID TREATMENT ON THE INCIDENCE OF LUNG METASTASIS IN C3H/HeN FEMALE MICE ASSOCIATED WITH OROTIC ACID TREATMENT

<table>
<thead>
<tr>
<th>No. animals</th>
<th>Treatment</th>
<th>No. with spontaneous mammary tumors</th>
<th>Av. age when tumor developed (mo.)</th>
<th>No. with lung metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>Orotic acid*</td>
<td>32 (94%)</td>
<td>15.1 ± 2.9</td>
<td>14 (44%)</td>
</tr>
<tr>
<td>37</td>
<td>none</td>
<td>30 (81%)</td>
<td>14.1 ± 3</td>
<td>3 (10%)</td>
</tr>
</tbody>
</table>

* 0.1 per cent orotic acid in the drinking water.

DISCUSSION

The pyrimidine, orotic acid, which has been shown to be an inhibitor of the carcinogenic process initiated by the chemical carcinogens urethan and methylcholangrene, did not significantly inhibit the carcinogenic process initiated by the viral agent associated with mouse mammary tumor.

The explanation for this difference in response may involve a time factor as well as a possible difference between the basic mechanisms of chemical and viral carcinogenesis. In the studies on urethan and methylcholangrene (2, 3) the carcinogen and the orotic acid were administered at the same time, as compared with an interval of 20 weeks between the ingestion of the milk agent and treatment with orotic acid in the present study. Similarly, a shorter time elapsed between the administration of the chemical carcinogens and the development of tumors. Urethan- and methylcholangrene-induced tumors developed in 8 weeks, whereas the average latent period for the mammary tumors was 44 weeks.

Of particular interest, however, was the observation that metastasis of mammary tumor to the lung was markedly increased in the mice receiving orotic acid. Because metastasis is one of the gravest problems associated with the clinical treatment of cancer, any situation that alters the metastatic process merits close scrutiny. There seem to be three ways by which an agent might facilitate the establishment of a metastatic lesion. One is by altering the host, another is by altering the tumor cells, and a third is by a combination of host and tumor alterations. A variety of agents have been shown to produce changes in the host which affect the incidence of metastasis. Substances or conditions which produce an effect on the host have been discussed by Wood et al. (4). Among them are steroid hormones, anti-coagulants, and radiation. There is little evidence that any agent has a direct effect on the tumor cell or increases the cell's capacity to metastasize.

In the present study the increased metastasis to the lung in the orotic acid-treated animals which received an intravenous injection of tumor cells would seem to indicate that the orotic acid was not producing its effect on the primary tumors in the first part of the study by increasing the release of tumor cell emboli from the primary tumor, but rather by increasing the likelihood that the tumor cells would become established at a metastatic focus. Aside from this observation the present study reveals nothing of the role of orotic acid in the metastatic process, but it is tempting to postulate that the availability of nucleic acid precursors may be a limiting factor in the nutritional re-

TABLE 2

EFFECT OF OROTIC ACID TREATMENT ON THE INCIDENCE OF LUNG METASTASIS IN C3H/HeN MICE GIVEN INJECTIONS INTRAVENOUSLY† OF C3H/HeN MAMMARY TUMOR CELLS

<table>
<thead>
<tr>
<th>No. MICE USED IN EACH GROUP</th>
<th>No. ANIMALS WITH LUNG METASTASIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
</tr>
<tr>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>

Total number of mice used: 49

Average incidence: 21 (43%) | 12 (18%)

* 0.5 per cent orotic acid in distilled water; 1.0 ml. subcutaneously daily ×5, then 0.5 ml. daily given 5 days per week.
† 375,000 mammary tumor cells injected intravenously into the tail vein.

Both the orotic acid-treated and control mice were killed 52 days after the intravenous tumor cell injection. Fifty-two days was selected as the appropriate time to sacrifice the animals, because other studies in our laboratory had shown that no appreciable change in the number of animals that would develop lung metastases occurred after 42 days. The autopsies were performed and the tissues were prepared and examined in the same manner as in the spontaneous tumor study.

RESULTS

Gross and microscopic examinations of all the organs of both experimental and control animals revealed metastatic tumors only in the lungs. The histological features of these metastatic tumors were comparable to the respective donor tumors.

Part I.—The results of this study are summarized in Table 1. Thirty-two of the 34 animals treated with orotic acid developed mammary tumors (94 per cent) with an average latent period of 15.1 months. Of the 37 control animals, 30 developed mammary tumors (81 per cent) at an average latent period of 14.1 months. These differences in incidence and latent period are not significant.

Fourteen (44 per cent) of the orotic acid-treated animals had foci of mammary tumor metastasis, whereas three (10 per cent) of the control animals had tumor metastasis in their lungs. This difference in the incidence of lung metastasis in the orotic acid-treated and control group is significant by the X² test at the 0.01 level.

Part II.—Of the 49 animals receiving both orotic acid and intravenous tumor cell injections, 21 showed microscopic lung metastases (42.8 per cent); of the 68 control animals receiving only tumor cells intravenously, twelve showed microscopic lung metastasis (17.6 per cent), (Table 2). This difference is significant by the X² test at the 0.01 level.
quirements for the growth of tumor cells, particularly in the potentially adverse environment of their intravascular situation in a distant organ.

REFERENCES


Effect of Orotic Acid on the Metastasis of Mammary Tumors in Mice

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