Neoplastic Response of Various Tissues to the Systemic Administration of the 8-Methyl Ether of Xanthurenic Acid

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SUMMARY
Following the repeated s.c. administration of an aqueous solution of the 8-methyl ether of xanthurenic acid into female Swiss mice, an incidence of 23% malignant tumors of the lymphoreticular system was observed. This incidence was significantly greater than the one of 4% found in a corresponding control group. Although other tumors — mammary adenoma, pulmonary adenoma, uterine fibroadenoma, etc. — were observed in both groups, no significant difference in incidence was apparent.

INTRODUCTION
The genesis of hematologic malignancies in mice following systemic administration of certain metabolites of the essential amino acid tryptophan has been reported (9-11, 15, 16). Indole (10, 15, 16), 3-indoxyl sulfuric acid (indican) (11), 3-indoleacetic acid (15, 16), 3-hydroxyanthranilic acid (9, 16) and 2-aminoacetophenone (16) acted as leukemogenic agents. Carcinomas of the urinary bladder were observed in mice following the local application of certain urinary metabolites of tryptophan (3). The 8-methyl ether of xanthurenic acid (XAE), xanthurenic acid, 8-hydroxyquinidic acid, 3-hydroxy-L-kynurenine, and 3-hydroxyanthranilic acid were associated with the production of an augmented, significant incidence of bladder carcinomas (3). The greatest incidence of carcinomas occurred following exposure to XAE (3). These bladder carcinomas could also be produced by placing a foreign body pellet of cholesterol into the bladder lumen and subsequently administering XAE s.c. (7). These data suggested that XAE might also be carcinogenic for other tissues, in addition to the urinary bladder, if administered systemically. This report presents the incidence of tumors in organs other than the urinary bladder following the s.c. injection of XAE.

MATERIALS AND METHODS
The 4 groups of female Swiss mice studied in the experiment were described previously (7) (Table 1). The mice in Group 1 had pellets of cholesterol surgically placed into their urinary bladders 90-100 days before the initiation of s.c. injections of XAE (7). Group 2 received s.c. injections of XAE but had no pellets placed in their bladders. Group 3 received s.c. injections of the aqueous solvent employed to dissolve XAE, and Group 4 served as untreated controls. The mice in all groups were the same age and size, and received s.c. injections during the same period. The 1st injection was administered when the mice were about 6 months old.
XAE was prepared as described by Price and Dodge (14). It was dissolved with a few drops of 7.16 N NH₄OH and diluted

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice started on experiment</th>
<th>No. of mice evaluated</th>
<th>Average life span (days)</th>
<th>Lymphoreticular</th>
<th>Mammary adenoma</th>
<th>Pulmonary adenoma</th>
<th>Uterine fibroadenoma</th>
<th>Other</th>
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</thead>
<tbody>
<tr>
<td>8-Methyl ether of xanthurenic acid</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>38</td>
<td>23</td>
<td>593</td>
<td>4</td>
<td>1</td>
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<td>0</td>
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<td>42</td>
<td>21</td>
<td>606</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>4</td>
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<tr>
<td>Total</td>
<td>80</td>
<td>44</td>
<td>600</td>
<td>10 (22.7%)</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1b</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>42</td>
<td>22</td>
<td>555</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>24</td>
<td>500</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1b</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>46</td>
<td>550</td>
<td>2 (4.3%)</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1b</td>
</tr>
</tbody>
</table>

Survival of mice and incidence of tumors following repeated s.c. injections of the 8-methyl ether of xanthurenic acid.

* Ovarian Tumor.
* Subcutaneous Fibroma.

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with 2 × 10⁻² M phosphate buffer (pH 7.4) to a final concentration of 1 mg of XAE/ml of solution. One ml of this solution was administered s.c. to the mice in Groups 1 and 2 three times each week for a period of 44 weeks (132 mg of test compound/animal). In a similar manner, the aqueous buffer containing a few drops of NH₄OH was administered to the animals in Group 3.

All animals dying prior to the completion of the series of injections were discarded from the experiment. Mice surviving for the full period of the injections, and those living until the end of the experimental period (22 months) were inspected carefully at autopsy. All external, thoracic, or abdominal tissues suggestive on gross examination of neoplastic growth were prepared, stained with hematoxylin and eosin, and inspected microscopically. The incidence of tumors in those mice receiving injections of XAE (Groups 1 and 2) was compared statistically with the incidence of tumors observed in those mice not exposed to injections of XAE (Groups 3 and 4) by the exact method for 2 × 2 tables (12). These data are presented in Table 1.

RESULTS

The incidence of malignant tumors of the lymphoreticular system observed in the groups exposed to XAE was significantly greater (P < 0.01) than that of the control groups. These lesions were histologically like those reported by other investigators (8, 13, 17), and seemed to involve the thymus gland in most cases. Additionally, involvement of the mediastinal lymph nodes, the spleen, kidneys, small intestine, and urinary bladder was frequently noted. The incidence of bladder carcinomas observed in the 4 groups was reported previously (7). Seven mice in Group 1 developed bladder carcinomas, and 3 of these 7 mice also had malignant tumors of the lymphoreticular system. No bladder carcinomas were found in the mice comprising Group 2, but malignant tumors of the lymphoreticular system were present in 6 of these mice. The mice in Groups 3 and 4 failed to develop bladder carcinoma and the incidence of malignant tumors of the lymphoreticular system was only 4.3%. The incidence of tumors present in other organs was not significantly different in the treated and control groups (Table 1).

DISCUSSION

These data suggest that XAE may stimulate the formation of tumors in tissues other than the urinary bladder if administered systemically. The incidence of malignant tumors of the lymphoreticular system observed in these experimental groups following exposure to XAE is comparable to that reported (9-11, 15, 16) for several other metabolites of tryptophan. Other tissues did not appear to be sensitive to the carcinogenic activity of XAE (Table 1). Thus, in mice, 2 tissues—the urinary bladder and the lymphoreticular system—seem responsive to the carcinogenic influences of XAE.

When ¹⁴C-labeled XAE was injected s.c. or i.p. into the mouse (5) only one additional metabolite, present in the urine in very small quantities, could be detected (G. T. Bryan and G. M. Lower, Jr., unpublished observations). Almost all of the administered doses were recovered in the urine as unmetabolized XAE. However, other metabolites of tryptophan that have been tested for leukemogenic activity are not metabolically inert. For example, it was observed that 3-hydroxyanthranilic acid and 3-hydroxy-L-kynurenine are metabolized rapidly after i.p. injection into the mouse (6). After labeled XAE was injected intraluminally into the urinary bladder of the mouse, at the end of the 24-hr experimental period almost all of the radioactivity had passed across the urinary bladder and was present in the carcass (5). Thus, XAE seems to be widely distributed in the tissues of the mouse under the experimental conditions employed to detect carcinogenic activity. Since it is apparently metabolically inactive to a great extent, XAE may participate directly in the process of carcinogenesis.

The mechanism of carcinogenesis by XAE is unknown (4). When XAE is applied locally to the urinary bladder or injected s.c., at least one additional factor must be present in the bladder for the subsequent development of bladder carcinomas (7). The observed carcinogenic effect on the lymphoreticular system is of interest because the mice were about 6 months old at the time of the 1st injection of XAE. Radiation is also leukemogenic in adult mice, but conversely, urethan is leukemogenic only when treatment is begun shortly after birth (1). The thymus gland of older mice may also be sensitive to the carcinogenic stimuli of chemicals, for Brues and Marble (2) observed the formation of thymic lymphoblastoma in mice 10-20 weeks of age at the beginning of skin painting with a carcinogenic tar. It is probable, however, that a higher incidence of malignant tumors of the lymphoreticular system might occur if younger mice were exposed to systemically administered XAE.

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