The Effect of a Series of Ethylenimine Derivatives on Myeloid Chloroleukemia in the Rat

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With the technical assistance of Lawrence Sebastianelli

Triethylene melamine and other ethylene substituted aminotriazines have been shown to retard the growth of a number of experimental tumors in our laboratory1, 2 and elsewhere.3-11 Since promising results have been obtained clinically11-14 with certain of these compounds, these studies were extended to include additional compounds* possessing one or more ethylenimine groupings in their structure. There have been a number of reports from several laboratories on the marked carcinolytic effect of X-ethylene substituted phosphoramides against a variety of experimental tumors including mouse leukemias.15-19 The purpose of this paper is to report the activity of certain compounds of this type against myeloid chloroleukemia in the rat.

The leukemia studied was a myeloid chloroleukemia which was induced by Shay† by intra gastric administration of methylcholanthrene in rats.20, 21 It can only be transmitted to rats less than 8 days old by intraperitoneal injection of blood or a suspension of either liver or spleen. The leukemia is manifested in the animals approximately five or six weeks after “charging” with leukemic cells. The animals show a loss of weight, anemia, and porphyric tears. The white blood cell counts increase to 50,000 to 300,000 per cu.mm. and immature blood cells (myeloblasts, promyelocytes, and myelocytes) range from 20 to 40 per cent of the total. The white blood cell counts remain elevated and immature forms remain in the peripheral blood, although there is some fluctuation. There is a steady loss in weight until the death of the animal which occurs approximately nine days after the leukemia becomes evident as measured by the above criteria.

Autopsy shows that the spleen is enlarged to about twice its normal size. The liver and kidneys are very pale in color, and there are deposits of green pigment covering the brain, abdominal organs, and dorsal area of the peritoneal cavity and infiltrating the liver, mesentery, thymus, lymph nodes, and bone marrow. Successful transfers of this leukemia are obtained in 76 per cent of the rats and the incidence of spontaneous regression is less than 0.5 per cent. These observations are in accordance with, and supplement, the description by Shay.21

The characteristics of this leukemia suggested that it might be a useful screening tool for antileukemic substances. The data presented in this paper fully

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support this idea. A series of phosphoramides was studied and their relative activity against this leukemia is reported below.

**Experimental Methods**

On the day that the leukemic state was proved by the general appearance of the rats and elevated white blood cell counts, the animals were placed in groups of five, all approximately the same age and weight. Included in each experiment as controls were a leukemic saline-treated group and a nonleukemic saline-treated group. In addition, every experiment contained a group treated with phosphoric acid triethylenimide (TEPA) which served as a chemical control group with which the activity of any other chemical tested could be compared. The compounds were prepared in buffered saline and administered in a volume of 0.5 to 1.0 ml. Each was used at the level indicated in table 1. Treatment was started on the same day that the leukemia was demonstrated and the animals were injected intraperitoneally every other day until nine injections were administered. The course of the leukemic development was followed by daily observations of vitality and weight of the rats. White blood cell counts and differential smears were taken before treatment was started and then at two days and at approximately five day intervals thereafter. After treatment was stopped the rats were held until death.

**Table 1.—Effect of Ethylenimine Derivatives Against Myeloid Chloroleukemia**

<table>
<thead>
<tr>
<th>Chemical number</th>
<th>Chemical name</th>
<th>Conc. mg./Kg. body wt.</th>
<th>No. of animals in pooled experiments</th>
<th>Av. wt. in Gm. before treatment</th>
<th>Effect upon animals</th>
<th>Immature blood cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 days after initial treatment</td>
<td>8 days after initial treatment</td>
<td>Before treatment</td>
<td>2 days</td>
</tr>
<tr>
<td>3066C</td>
<td>Triethylenemelamine (TEM)</td>
<td>0.3</td>
<td>5</td>
<td>69 77 85</td>
<td>++ + ± - -</td>
<td>++ 16 to 50 per cent immature forms (myeloblasts, promyelocytes, myelocytes)</td>
</tr>
<tr>
<td>3155C</td>
<td>Phosphoric acid triethylenimide (TEPA)</td>
<td>3</td>
<td>60</td>
<td>62 69 84</td>
<td>++ + ± ± ±</td>
<td>+ 6 to 15 per cent immature forms (myeloblasts, promyelocytes, myelocytes)</td>
</tr>
<tr>
<td>3150C</td>
<td>N-Pentamethylene-N,N*-diethylenephosphoramide</td>
<td>4</td>
<td>15</td>
<td>61 72 85</td>
<td>++ + ± ± ±</td>
<td>± 1 to 5 per cent immature forms (myeloblasts, promyelocytes, myelocytes)</td>
</tr>
<tr>
<td>3172C</td>
<td>N,N'N*-Triethylenemethylenephosphoramide</td>
<td>8</td>
<td>15</td>
<td>64 77 82</td>
<td>++ + + ± ±</td>
<td>- normal differential</td>
</tr>
<tr>
<td>3173C</td>
<td>N-(3-Oxapentamethylene)-N,N'-diethylenephosphoramide</td>
<td>7.5</td>
<td>20</td>
<td>63 70 84</td>
<td>++ + - ± ±</td>
<td>-</td>
</tr>
<tr>
<td>3174C</td>
<td>Octyl N,N'-Diethylenediamidophosphate</td>
<td>80</td>
<td>15</td>
<td>65 68 81</td>
<td>++ + ± - -</td>
<td>-</td>
</tr>
<tr>
<td>3193C</td>
<td>N,N',N*-Triethylene-thiophosphoramide</td>
<td>5</td>
<td>25</td>
<td>68 75 87</td>
<td>++ ± ± ± -</td>
<td>-</td>
</tr>
<tr>
<td>Leukemic, Saline-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>++ + + + + + + + + +</td>
</tr>
<tr>
<td>Nonleukemic, Saline-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3066</td>
</tr>
</tbody>
</table>

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The following served as criteria of activity: lowering of white blood cell counts and reduction in the number of immature forms, improved general appearance and growth of the animals, and the per cent survival of chemically treated groups at the time 60 per cent of the leukemic saline-treated rats were dead. This figure was chosen because active compounds gave 100 per cent survival at this time. Average total survival time also gave additional criteria of activity.

**RESULTS**

As shown in table 1, seven compounds were found to be very active against this leukemia. The leukemic saline-treated controls all died with the symptoms and blood picture described above. The active compounds, however, caused the blood picture to approximate that of nonleukemic controls. Total blood counts dropped within the normal range and there was a marked reduction in the number of immature forms. The animals also resumed growth and regained their normal appearance. The improvement was evident within forty-eight hours and was complete within six days. The hematologic response is illustrated in figure 1, which represents an average response of the leukemic rats to the compounds tested. Each one of these compounds produced a rapid decrease of the total leukocyte count from 120,000 to below 20,000 per cu.mm. The average rate of disappearance of immature blood cells is represented in table 1.

The effect of these compounds on average survival time is illustrated in figure 2. Except for one compound (3172C) the survival time was increased by more...
than 100 per cent. That longer survival was not obtained with these compounds is due to the fact that treatment was stopped after seventeen days. Deaths were due to the recurrence of the leukemia. A number of these animals were autopsied and the gross pathology described above was found to be present.

**TABLE 1**

<table>
<thead>
<tr>
<th>Leukemic Saline-Treated</th>
<th>Phosphoric Acid Triethyleneimide (TEPA)</th>
<th>3 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>3155C</td>
<td>Triethylene Melamine</td>
<td>0.3 mg/kg</td>
</tr>
<tr>
<td>3173C</td>
<td>N-(3-Oxapentamethylene)-N',N'-Diethylene phosphoramid</td>
<td>7.5 mg/kg</td>
</tr>
<tr>
<td>3193C</td>
<td>N,N',N'-Triethyleneethiodiphosphamide</td>
<td>5 mg/kg</td>
</tr>
<tr>
<td>3198C</td>
<td>N-Pentamethylene-N',N'-Diethylene phosphoramid</td>
<td>4 mg/kg</td>
</tr>
<tr>
<td>3172C</td>
<td>N,N',N'-Tris(l-Methylenethylene) phosphoramid</td>
<td>80 mg/kg</td>
</tr>
<tr>
<td>3174C</td>
<td>Octyl N,N'-Diethylene diamidophosphate</td>
<td>80 mg/kg</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The myeloid chloroleukemia in the rat appears to be a useful tool for the study of antileukemic compounds. The incidence in charged rats is high, and clear-cut blood changes are obtained. When active agents are used, positive results are evident within a few days.

A series of N-ethylene substituted phosphoramides was found to be very effective in controlling this leukemia which has not responded to treatment with pteroylglutamic acid antagonists. The effectiveness of these phosphoramides was demonstrated by more detailed studies of the blood picture and general appearance of the animals than can ordinarily be obtained from studies in mice. Thus, additional information which may be of value can be obtained by using the rat to supplement the use of the mouse in screening compounds for antileukemic activity.

All of the compounds in table 1 effectively prolonged survival time. The phosphoramides are as effective as 3066C (TEM) and have the advantage of being considerably less toxic. Only compound 3172C failed to increase survival time more than 100 per cent. Two of the compounds (3173C and 3193C) are appreciably more stable and are less likely to polymerize in solution than are the rest of the compounds. Compound 3193C consistently gave a slightly more rapid response than the others.
A myeloid chloroleukemia in the rat has been studied and developed into an assay for the screening of antileukemic compounds.

A series of phosphoramide derivatives have been found to be effective against this leukemia, and comparative studies on their activity have been made. These compounds appear to be as effective as TEM and can be administered at higher dosage levels. All of the compounds produced a prompt remission of the disease.

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MYELOID CHLOROLEUKEMIA IN THE RAT

660


22 Our unpublished data.
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