Observations on the Antileukemic Activity of Pyridine-
2-carboxaldehyde Thiosemicarbazone
and Thiocarbohydrazone

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The investigation of the biological activity of thiosemicarbazones had as its origin Domagk's attempt to establish a relationship between the structure and antituberculous activity of a series of sulfathiadiazoles which were prepared by cyclizing the corresponding thiosemicarbazones (4). The end product, the thiadiazoles, proved uninteresting, but the intermediate thiosemicarbazones, which were tested by Behnisch and his group in Germany, were reported in 1946 to be strongly tuberculostatic in vivo (1, 4).

Interest in compounds of this type was stimulated by the report of Hamre and her group at the Squibb Institute for Medical Research that certain of the aromatic aldehyde thiosemicarbazones possessed in vivo activity against vaccinia virus—both in embryonated eggs and in mice (3, 5, 6). The antivaccinial activity of this class of compounds in mice has been confirmed and extended by R. L. Thompson and his co-workers (8, 9, 10, 11) and in this laboratory.

Compounds capable of inhibiting virus multiplication, which presumably involves synthesis of nucleoprotein, are of interest as candidate anticancer agents. Thiosemicarbazones have been tested, therefore, for activity against several solid tumors and leukemias in mice. Meta- and para-nitrobenzaldehyde thiosemicarbazones were found to have weak but consistent activity against Adenocarcinoma 755, restricting tumor growth to 40–50 per cent of that in untreated controls at levels that did not result in weight loss or death of treated animals. Thiosemicarbazones appear to be without appreciable activity against Sarcoma 180.

Pyridine-2-carboxaldehyde thiosemicarbazone (I) and the closely related pyridine-3-carboxaldehyde thiocarbohydrazone (II) were found to have activity against several lines of experimental leukemia.

![I](CH = N - NH - C - NH₃)

![II](CH = N - NH - C - NH - NH₂)

Thiosemicarbazone derivatives of the following aldehydes were tested against L1210 leukemia and found to be without significant effect in increasing the life span: p-acetamidobenzaldehyde, m- and p-nitrobenzaldehyde, p-nitrosalicylaldehyde, 5-nitrosalicylaldehyde, 2-furaldehyde, 2-thenaldehyde, pyrole-2-carboxaldehyde, indole-3-carboxaldehyde, pyridine-2,6-dicarboxaldehyde dithiosemicarbazone, quinoline-2-carboxaldehyde, quinoline-4-carboxaldehyde, and pyridoxal. Benzaldehyde thiosemicarbazone gave a 20 per cent increase in life span in preliminary screening experiments.

MATERIALS AND METHODS

In all experiments with L1210 leukemia, groups of ten DBA/2 mice were given inoculations intraperitoneally of approximately 1,000,000 leukemic cells, according to the technic of Law (7). Similarly, leukemia L4946 was inoculated in AKR mice. Dr. Burchenal of Sloan-Kettering Institute supplied the line of L82T used; this leukemia is...
transferred by inoculation into C58BLF1 mice. The drugs were administered intraperitoneally 24 hours after inoculation of leukemic cells, and treatment was continued on an alternate-day basis until death or for a total of ten injections. Exceptions to this standard treatment are indicated in footnotes to the tables. Animals were maintained on a stock diet except as indicated in those experiments where specific deficient diets were employed.

RESULTS

**L1210 leukemia.**—The effect of pyridine-2-carboxaldehyde thiosemicarbazone on L1210 leukemia is recorded in Table 1. These data show that this compound was capable of consistently increasing the life span of the leukemic mice to a moderate degree.

<table>
<thead>
<tr>
<th>DOSAGE (mg/kg)*</th>
<th>MEAN SURVIVAL TIME</th>
<th>PER CENT INCREASE IN LIFE SPAN†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (days)</td>
<td>Treated (days)</td>
</tr>
<tr>
<td>15</td>
<td>8.2</td>
<td>12.7</td>
</tr>
<tr>
<td>12</td>
<td>6.5</td>
<td>8.6</td>
</tr>
<tr>
<td>10</td>
<td>7.5</td>
<td>9.5</td>
</tr>
<tr>
<td>9</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>9.2</td>
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<td>7.0</td>
<td>10.0</td>
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<td>6.3</td>
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<td></td>
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<td>8.5</td>
</tr>
<tr>
<td>5</td>
<td>7.5</td>
<td>9.4</td>
</tr>
<tr>
<td>20</td>
<td>6.4</td>
<td>8.8</td>
</tr>
<tr>
<td>7.2</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7.2</td>
<td>10.1</td>
</tr>
<tr>
<td>6.4</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>6.9</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6.9</td>
<td>10.2</td>
</tr>
</tbody>
</table>

* The drug was administered intraperitoneally every other day until death, except where otherwise indicated.
† The standard deviation and Student’s “t” value were calculated for each experiment. The probability of the effect observed occurring by chance was <0.001 except where otherwise indicated.
‡ P = <0.01.

**L4946 and L82T leukemia.**—Data on the effect of pyridine-2-carboxaldehyde thiosemicarbazone on L4946 leukemia and of the corresponding thio-carbohydrazone on L82T are not so extensive as that on L1210 leukemia. That the drugs did significantly prolong the life of mice with these leukemias is shown by the data recorded in Table 3.

**TABLE 2**

<table>
<thead>
<tr>
<th>DOSAGE (mg/kg)*</th>
<th>MEAN SURVIVAL TIME</th>
<th>PER CENT INCREASE†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (days)</td>
<td>Treated (days)</td>
</tr>
<tr>
<td>60</td>
<td>7.2</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>6.4</td>
<td>8.3</td>
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<tr>
<td>50</td>
<td>6.3</td>
<td>8.3</td>
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<tr>
<td></td>
<td>6.7</td>
<td>7.9</td>
</tr>
<tr>
<td>7.0</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>7.2</td>
<td>9.6</td>
</tr>
<tr>
<td>25</td>
<td>6.3</td>
<td>7.3</td>
</tr>
<tr>
<td>75</td>
<td>6.4</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>8.0</td>
</tr>
<tr>
<td>60</td>
<td>6.4</td>
<td>8.8</td>
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<tr>
<td></td>
<td>6.7</td>
<td>8.5</td>
</tr>
<tr>
<td>25</td>
<td>6.7</td>
<td>9.1</td>
</tr>
<tr>
<td>40</td>
<td>6.7</td>
<td>8.6</td>
</tr>
</tbody>
</table>

* The drug was administered intraperitoneally every other day except where otherwise noted.
† The standard deviation and Student’s “t” value were calculated for each experiment. The probability was <0.001 except where otherwise noted.
‡ P = <0.01.
# Subcutaneous every other day.
Intraperitoneal every day.

The closely related compound, pyridine-2-carboxaldehyde thio-carbohydrazone, was found to be active against L1210 leukemia, as shown by the data summarized in Table 2. This compound was less toxic to mice than the corresponding thiosemicarbazone and was effective in increasing the life span of the leukemic animals at levels below the maximum tolerated dose.

**L1210 and L4946 leukemia.**—Pyridine-2-carboxaldehyde thiosemicarbazone and the isomeric nicotinaldehyde and isonicotinaldehyde thiosemicarbazones were observed to be completely without effect on the life span of mice given inoculations of leukemias L1210 and L4946. Isomers were also noted. The maximum tolerated dose of pyridine-2-carboxaldehyde thiosemicarbazone was 15 mg/kg compared with 75 mg/kg for pyridine-3-carboxaldehyde thiosemicarbazone, and 500 mg/kg for pyridine-4-carboxaldehyde thiosemicarbazone.

Marked differences in the toxicity of these isomers were also noted. The maximum tolerated dose of pyridine-2-carboxaldehyde thiosemicarbazone was 15 mg/kg compared with 75 mg/kg for pyridine-3-carboxaldehyde thiosemicarbazone, and 500 mg/kg for pyridine-4-carboxaldehyde thiosemicarbazone.

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4, show that the growth of L1210 tumor was restricted to 50 per cent of that of untreated controls. Growth of leukemic tumor L4946 could be restricted to 25–30 per cent of that of the controls by pyridine-2-carboxaldehyde thiosemicarbazone or the corresponding thio-carboxyhydrzone.

Attempts to potentiate the antileukemic effect of pyridine-2-carboxaldehyde thiosemicarbazone.—Microbiological studies showed that the inhibition of the thiosemicarbazone inhibition of E. coli, namely, ethionine, neopyrithiamine, and neopyrithiamine on a B1-deficient diet,4 were used in combinations with pyridine-2-carboxaldehyde thiosemicarbazone. None of these combinations appeared to be significantly more effective against L1210 than the thiosemicarbazone alone. Pyridine-2-carboxaldehyde thiosemicarbazone was not significantly more effective against L1210 leukemia in mice on a B1-deficient diet than it was in mice on a stock diet.

**TABLE 3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leukemia</th>
<th>Mean Survival Time</th>
<th>Per Cent Increase in Life Span</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridine-2-carboxaldehyde thiosemicarbazone</td>
<td>L4946</td>
<td>10</td>
<td>13.5</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
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<td>5</td>
<td>13.5</td>
<td>14.7</td>
</tr>
<tr>
<td>Pyridine-2-carboxaldehyde thiosemicarbazone</td>
<td>L82T</td>
<td>75</td>
<td>9.3</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>10.4</td>
<td>13.7</td>
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<tr>
<td></td>
<td></td>
<td>50</td>
<td>9.3</td>
<td>11.8</td>
</tr>
</tbody>
</table>

* Not significant.

**TABLE 4**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Tumor</th>
<th>Dosage (mg/kg)</th>
<th>Av. Mouse Wt. Change (gm.)</th>
<th>Mortality</th>
<th>Av. Tumor Wt. (mg.)</th>
<th>Tumor Weight Per Cent of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>L1210</td>
<td></td>
<td>+1.1</td>
<td>0/10</td>
<td>475.5</td>
<td></td>
</tr>
<tr>
<td>Pyridine-2-carboxaldehyde thiosemicarbazone</td>
<td>L1210</td>
<td>8‡</td>
<td>-0.8</td>
<td>2/10</td>
<td>234.8</td>
<td>49‡</td>
</tr>
<tr>
<td>Pyridine-2-carboxaldehyde thiosemicarbazone</td>
<td>L1210</td>
<td>25§</td>
<td>-0.8</td>
<td>0/10</td>
<td>250.6</td>
<td>53§</td>
</tr>
<tr>
<td>Control</td>
<td>L4946</td>
<td></td>
<td>+2.8</td>
<td>0/10</td>
<td>772.9</td>
<td></td>
</tr>
<tr>
<td>Pyridine-2-carboxaldehyde thiosemicarbazone</td>
<td>L4946</td>
<td>5§</td>
<td>+0.1</td>
<td>0/10</td>
<td>391.7</td>
<td>51</td>
</tr>
<tr>
<td>Pyridine-2-carboxaldehyde thiosemicarbazone</td>
<td>L4946</td>
<td>10§</td>
<td>-3.1</td>
<td>2/10</td>
<td>97.6</td>
<td>25</td>
</tr>
<tr>
<td>Pyridine-2-carboxaldehyde thiosemicarbazone</td>
<td>L4946</td>
<td>30§</td>
<td>+0.5</td>
<td>2/10</td>
<td>258.8</td>
<td>51</td>
</tr>
</tbody>
</table>

* P = <0.001, except where otherwise indicated.
‡ The drug was administered on the 1st, 3rd, and 5th days after tumor implantation; tumors were excised and weighed on the 7th day.
§ P = <0.01.
§ The drug was administered on the 1st, 3rd, 5th, and 7th days after tumor implantation; tumors were excised and weighed on the 8th day.

Escherichia coli by pyridine-2-carboxaldehyde thiosemicarbazone could be prevented by methionine, homocysteine, thiamine (B1), and, to a lesser degree, by other metabolites. Attempts to reverse the toxicity and the antileukemic activity of pyridine-2-carboxaldehyde thiosemicarbazone with these agents were not successful.

Attempts were made to potentiate the antileukemic effect of this compound on L1210 with several known antileukemic agents including A-methopterin, 6-mercaptopurine, and azaserine (O-diazoacetyl-L-serine). Such combinations gave no potentiation of antileukemic activity. In addition, analogs of the compounds found to reverse the thiosemicarbazone inhibition of E. coli, namely, ethionine, neopyrithiamine, and neopyrithiamine on a B1-deficient diet,4 were used in combinations with pyridine-2-carboxaldehyde thiosemicarbazone. None of these combinations seemed to be significantly more effective against L1210 than the thiosemicarbazone alone. Pyridine-2-carboxaldehyde thiosemicarbazone was not significantly more effective against L1210 leukemia in mice on a B1-deficient diet than it was in mice on a stock diet.

**DISCUSSION**

There are several features of interest about the antileukemic activity of these simple derivatives of a-picolinaldehyde: (a) position isomers of pyridine-2-carboxaldehyde thiosemicarbazone are devoid of antileukemic activity, thus suggesting a rather high degree of structural specificity; (b) the thiosemicarbazone and thio-carboxyhydrzone derivatives have exhibited activity against three different types of leukemia: L1210, L4946, and L82T. The latter two were not significantly more effective against L1210 leukemia in mice on a B1-deficient diet.

In two experiments, neopyrithiamine was observed to have a statistically significant effect on L1210 leukemia in mice on a B1-deficient diet.
ent lines of leukemia—L1210, L4946, and L82T; 
(c) structurally, these compounds are unlike any 
of the agents known to be effective in increasing 
the life span of mice with experimental leukemias. 
For these reasons the antileukemic activity of 
these compounds has been considered worthy of 
interest.

No real leads are yet evident to suggest a mode 
of action of pyridine-2-carboxaldehyde thiosemi-
carbazone.

SUMMARY

1. Pyridine-2-carboxaldehyde thiosemicarba-
zone and the corresponding thiocarbohydrazone 
consistently and significantly increased the life 
span of mice with L1210 leukemia.

2. Less extensive experiments showed that treat-
ment of mice inoculated with L4946 and L82T 
leukemias with these compounds resulted in sta-
tistically significant increases in life span.

ACKNOWLEDGMENTS

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