57. Effect of Tropolone and Related Compounds on Yoshida Sarcoma (3rd Report).

Especially in Regard to p-Aminoderivatives of Tropolone and Hinokitiol.

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(Comm. by T. Kumagai, M.J.A., May 16, 1951.)

p-Aminotropolone was found as an effective substance in our experiment and its mode of action to mitoses was reported in the 2nd report\(^1\). (Fig. 1).

In this report the amino-derivatives of tropolone, hinokitiol and α-thujaplicin were studied.
The substances examined are as follows (name, molecular formula, m.p. and structure).

(1) O-Acetyl-o-amino-hinokitiol, C\(_{12}\)H\(_{16}\)O\(_2\)N, m.p. 72°, (A)\(^9\).
(2) p-Amino-α-thujaplicin, C\(_{13}\)H\(_{15}\)O\(_2\)N, m.p. 174°, (B)\(^3\).
(3) p-Amino-hinokitiol, C\(_{10}\)H\(_{13}\)O\(_2\)N, m.p. 131°, (C)\(^9\).
(4) o'-Bromo-p-amino-hinokitiol methylether α, C\(_{11}\)H\(_{15}\)O\(_2\)NBr, m.p. 210°, (D or E)\(^3\).
(5) o'-Bromo-p-amino-hinokitiol methylether β, C\(_{11}\)H\(_{15}\)O\(_2\)NBr, m.p. 165°, (E or D)\(^3\).
(6) p-Aminotropolone\(^9\) hydrochloride.
(7) o-Bromo-p-aminotropolone\(^3\) kaliumsalt.

Fig. 1. Sarcoma cells in the peritoneal fluid of the rat, 3 days after i.p. inoculation, 3 hours after i.p. injection of p-aminotropolone (20 mg. per 100 g. body weight) Giemsa's stain.
(8) \( p \)-Aminotropolone diacetate, \( \text{C}_9\text{H}_9\text{O}_4\text{N} \), m.p. 181.5°; (F)\(^9\).
(9) \( o \)-Bromo-\( p \)-aminotropolone diacetate, \( \text{C}_9\text{H}_9\text{O}_4\text{NBr} \), m.p. 167°, (G)\(^7\).

Technic and assays of the effect on tumors cells: were the same as in the 1st report.

(1) \( O \)-Acetyl-\( o \)-aminohinokitiol: 20 mg. of it was injected intraperitoneally. There appeared no remarkable changes to mitoses.

(2) \( p \)-Amino-\( \alpha \)-thujaplicin: 20 mg. of it was dissolved in 40% propyleneglycol. As it was impossible to dissolve it clearly, it was injected intraperitoneally as a suspension. Characteristic mitotic changes were not obtained.

(3) \( p \)-Aminohinokitiol: 20 mg. of it dissolved in 20% propyleneglycol was injected intraperitoneally. Though the mitotic cells decreased in the microscopical visual field, ones observed with difficulty were almost intact. The resting cells were not greatly injured and the cellular reactions were observed relatively.

(4) \( o' \)-Bromo-\( p \)-aminohinokitiol methylether \( \alpha \):

(5) \( o' \)-Bromo-\( p \)-aminohinokitiol methylether \( \beta \):

10 mg. of (4) and (5) dissolved in 50% propyleneglycol were injected intraperitoneally. There appeared no significant changes in chromosomes.

(6) \( p \)-Aminotropolone hydrochloride: 20 mg. of it was contained in 1 c.c. solution. As the peritoneal fluid increased highly by the intraperitoneal injection of this HCl-salt, it was unsuitable for observation.

(7) \( o \)-Bromo-\( p \)-aminotropolone K-salt: 1 c.c. solution containing 20 mg. of it was prepared. As the colour of the solution became
dark gradually, the quality of it might be changed and the effect to mitoses was scarcely noticed.

(8) \( p \)-Aminotropolone diacetate: In cases injected with 40 mg. of it dissolved in 20\% propylene glycol this dose was thought as M.T.D. as a point of view of the general condition of animals. The remarkable decrease of mitoses appeared and in the few remaining mitotic cells there showed scatterings, fragmentations and aggregations of chromosomes in metaphase for the most part but a few intact mitotic cells existed also. Though the damage to the resting cells was not so remarkable, there appeared caryorrhexes, achromasies and hypochromasies in a part of them. The changes continued till 48 hours after injection but the prolongation of life was not achieved.

In cases injected with 20 mg. of it dissolved in 20\% propylene glycol no great difference was observed, compared with 40 mg. injected cases. The mitotic cells began to appear again 24 hours after injection. No remarkable changes in chromosomes were noticed in cases injected with 10 mg. of it.

(9) \( o \)-Bromo-\( p \)-aminotropolone diacetate: Although the remarkable decrease of mitoses and the damaged figures in metaphase (fragmentations, scatterings and aggregations) were observed by injection of 20 mg. of it dissolved in 20\% propylene glycol per 100 g. body weight intraperitoneally, the rats died 48 hours after injection. In cases injected with 10 mg, 5 mg and 2 mg not only the decrease of the mitotic cells but also the damage to the resting cells were noticed markedly and so these doses were unsuitable for observation.

In cases injected with 1 mg. dissolved in 10\% propylene glycol intraperitoneally the interruption of the mitotic process in metaphase (Table I and Table II) and fragmentations, scatterings, ag-

<table>
<thead>
<tr>
<th>Phase</th>
<th>Before treatment</th>
<th>1 hour after treatment</th>
<th>3 hours</th>
<th>6 hours</th>
<th>9 hours</th>
<th>12 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophase</td>
<td>20</td>
<td>2</td>
<td>7</td>
<td>16</td>
<td>13</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Metaphase</td>
<td>Not damaged</td>
<td>37</td>
<td>14</td>
<td>20</td>
<td>24</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Damaged</td>
<td>0</td>
<td>63</td>
<td>64</td>
<td>52</td>
<td>57</td>
<td>73</td>
</tr>
<tr>
<td>Anaphase</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Telophase</td>
<td>35</td>
<td>13</td>
<td>4</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>20</td>
</tr>
</tbody>
</table>
gregations and escapes of chromosomes in metaphase (Fig. 2) were observed, though a few mitotic cells remained uninjured. There appeared also melts of protoplasm in about 20% of the resting cells. The many mitotic cells began to appear again 24 hours after injection, the prolongation of life was not acquired and the tissue invasion of tumor cells was remarkable.

Table II.

Table II. The number of dividing cells of each stage as counted from 1000 sarcoma cells (The averaged number in the same two cases as Table I).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Before treatment</th>
<th>1 hour after treatment</th>
<th>3 hours</th>
<th>6 hours</th>
<th>9 hours</th>
<th>12 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophase</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Metaphase (damaged)</td>
<td>11 (0)</td>
<td>20 (16)</td>
<td>11 (10)</td>
<td>17 (13)</td>
<td>37 (29)</td>
<td>52 (44)</td>
<td>10 (4)</td>
</tr>
<tr>
<td>Anaphase</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Telophase</td>
<td>14</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

Fig. 2. Sarcoma cells in the peritoneal fluid of the rat, 3 days after i.p. inoculation, 9 hours after i.p. injection of o-Bromo-p-aminotropolone diacetate (1 mg. per 100 g. body weight) Giemsa's stain.

Summary and Conclusion.

9 substances were examined especially in regard to the p-amino-derivatives of tropolone and hinokitiol in this report.

1) Isopropyl group at m-position in hinokitiol may be the cause why the characteristic changes of chromosomes were not observed in cases injected with the substances in relation to p-amino-hinokitiol.

2) o-Bromo-p-aminotropolone was the most effective of the substances examined till now. There appeared the similar changes
to the so-called colchicine effect and moreover the ratio between the minimum lethal dose of the animal and the minimum effective dose to Yoshida sarcoma cells was high.

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