Teratogenic Actions of Some Methylated Xanthines in Mice

By

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Introduction

Teratogenicity of caffeine injected into pregnant mice with a fairly high dosage has been established by Nishimura and Nakai ('60) and Nakai ('61). Knoche and König ('64) confirmed similar effect of oral administration of this compound to pregnant mice. Recently, Georges and Denef ('68) reported on limb malformations induced by aminophylline in rats and suggested a specific action of the xanthine nucleus by the fact that the abnormalities occur with a peculiar frequency in the left posterior limb. Theophylline and theobromine as well as caffeine are commonly used for therapeutic purposes. The main purpose of the present experiments is to determine whether or not theophylline and theobromine show the teratogenic action in mouse embryos.

Methods

SPF mice of the ICR-JCL strain at 3 weeks of age were purchased from Japan CLEA Co. (Takatsuki, Osaka) and maintained until 8 weeks of age in the animal room of the Research Laboratories. Then, each virgin female was placed overnight with a male of the same age. Copulation was established by the presence of the vaginal plug next morning, and this day was tentatively designated as day zero of gestation. Animals were kept on the compressed chow (Japan CLEA Co.) and fresh tap water ad libitum. Experiments were carried out in the animal room where the temperature (22 ± 1°C) and humidity (55 ± 5%) were maintained.
Theophylline (J.P.), theobromine (J.P.) theobromine sodium salicylate (Wako Pure Chemical Industries Ltd.) or caffeine (J.P.) was administered once to the mice on day 12 of gestation by means of intraperitoneal injection. The concentration of each compound was regulated for a use of 10 ml/kg in each mother. The control mothers received a treatment with the same volume of 0.5% CMC suspension in the same way as in the experimental groups.

Results and Discussion

A. Theophylline experiment

Theophylline (solubility: 1g/120 ml) suspended in 0.5% CMC suspension was administered into three groups at the doses of 175 mg/kg (TP-175), 200 mg/kg (TP-200) or 225 mg/kg (TP-225) respectively. The results are summarized in Table 1A.

In the groups TP-175 and TP-200, most mothers showed slight dyspnea and convulsion for several minutes after treatment. In the group TP-225, such toxic signs were severer and about 40% of the mothers were found dead in the next morning of treatment. Malformed fetuses and fetuses with subcutaneous hematoma were found in all the treated groups.

The hematoma was observed in most cases at the upper and/or lower jaw and occasionally in the digital regions. It is to be noticed that the hematoma was accompanied often by some adjacent skeletal defects.

Among the treated groups, a dose effect responses is demonstrated on both teratogenicity and occurrence of subcutaneous hematoma. The main type of malformations was skeletal defects. The cleft palate occurred most often and the incidence of digital defects or macrognathia followed to that. The frequency of digital abnormalities classified according to their site is shown in Table 1B.

A left side preponderance of these abnormalities is likely to exist in both fore- and hind limbs in the groups TP-200 and TP-225.

Mortality of the offspring was significantly higher in the group TP-225 than that in the control group.

Average body weight of the live fetuses was significantly lowered in the groups TP-200 and TP-225 as compared with that of the control.
Table 1A  Effects of administration of theophylline (TP) given on gestational day 12 on mouse embryos

<table>
<thead>
<tr>
<th>Group (mg/kg)</th>
<th>No. of mothers treated</th>
<th>No. of maternal death</th>
<th>Total implants</th>
<th>No. of resorptions out of total implants (%)</th>
<th>Average litter size ± SX (g)</th>
<th>Average body weight ± SX (g)</th>
<th>No. of malformed and their type* (%: out of total live)</th>
<th>No. of subcutaneous hematoma and their site* (%: out of total live)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP-175</td>
<td>25</td>
<td>0</td>
<td>317</td>
<td>38 (12.0)</td>
<td>11.2</td>
<td>1.35 ± 0.007</td>
<td>33* (11.8) CP 32  DD 3  MG 1  UH 1</td>
<td>1  HD 5 (1.8) HF 1</td>
</tr>
<tr>
<td>TP-200</td>
<td>26</td>
<td>0</td>
<td>317</td>
<td>33 (10.4)</td>
<td>10.9</td>
<td>1.31* ± 0.009</td>
<td>44* (15.5) CP 43  DD 4  MG 3</td>
<td>16* HD 3 (5.6) HF 14</td>
</tr>
<tr>
<td>TP-225</td>
<td>26</td>
<td>11</td>
<td>196</td>
<td>56* (28.6)</td>
<td>9.3</td>
<td>1.34** ± 0.011</td>
<td>65* (46.4) CP 64  DD 16  MG 16  OE 2</td>
<td>38* HD 12 (27.1) HF 37</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>0</td>
<td>265</td>
<td>25 (9.4)</td>
<td>12.0</td>
<td>1.37 ± 0.008</td>
<td>0 -</td>
<td>0 -</td>
</tr>
</tbody>
</table>

*: Significant at P<0.01
**: Significant at P<0.05

* Code for type and site of abnormalities
  CF: Club foot  OE: Open eyelids
  CP: Cleft palate  UH: Umbilical hernia
  DD: Digital defect  HD: Hematoma at digits
  MG: Micrognathia  HF: Hematoma on face
Table 1B Frequency of digital abnormalities classified according to their site

<table>
<thead>
<tr>
<th>Group</th>
<th>Forelimb</th>
<th>Hind limb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>TP-175</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TP-200</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>TP-225</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

B. Theobromine experiment

Theobromine (solubility: 1g/2000 ml) suspended in 0.5% CMC suspension or theobromine sodium salicylate solubilized in saline was injected as is shown in Table 2A.

In the groups TB-500 and TB-600, the mothers showed twitching of the abdominal muscle for a few minutes and decreased bodily mobility for several minutes after the treatment. In the group TB-600, about 40% of the mothers were found dead in the next morning of treatment. In the group TSS-600, the mothers showed increased irritability for a few minutes after injection and were then followed by decreased mobility for several minutes. About one-fourth of the animals were found dead in the next morning.

In Table 2A, it is shown that fetuses with skeletal malformations and/or subcutaneous hematomas were found in all the treated groups. Between the groups TB-500 and TB-600, a dose effect response is shown with respect to the teratogenicity. In the groups TB-500 and TB-600, the digital defects were most frequently found while in the group TSS-600 the incidence of cleft palate was the highest.

In all the treated groups, the hematoma was found most often at the upper and/or lower jaw and occasionally in the digital regions. Like in the theophylline experiment, the hematoma was often accompanied by some adjacent skeletal defects.

The frequency of digital abnormalities classified according to their site is shown in Table 2B.

The similar tendency shown in Table 1B is found in the groups with those compounds.

Mortality of the offspring was significantly higher in the group TB-600 than that of the control group.
Table 2A  Effects of administration of theobromine (TB) and theobromine sodium salicylate (TSS) given on gestational day 12 on mouse embryos

<table>
<thead>
<tr>
<th>Group (mg/kg)</th>
<th>No. of mothers treated</th>
<th>No. of maternal death</th>
<th>Total implants</th>
<th>No. of resorption out of total implants (%)</th>
<th>Average litter size</th>
<th>Average body weight ± SE (g)</th>
<th>No. of malformed and their type* out of total live (%)</th>
<th>No. with subcutaneous hematoma and their site* out of total live (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB-500</td>
<td>20</td>
<td>0</td>
<td>244</td>
<td>32 (13.2)</td>
<td>10.6</td>
<td>1.32* 0.008</td>
<td>20* CP 3 (9.4) DD 16</td>
<td>24* HD 15 (11.3) HF 21</td>
</tr>
<tr>
<td>TB-600</td>
<td>37*</td>
<td>14</td>
<td>198++</td>
<td>72* (36.4)</td>
<td>7.0</td>
<td>1.27* 0.012</td>
<td>19* CP 5 (15.1) DD 17</td>
<td>18* HD 10 (14.3) HF 13</td>
</tr>
<tr>
<td>TSS-600</td>
<td>25</td>
<td>6</td>
<td>259</td>
<td>33 (12.7)</td>
<td>11.9</td>
<td>1.33* 0.007</td>
<td>36* CP 4 (14.6) DD 17 MG 6</td>
<td>23* HD 12 (10.2) HF 20</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>0</td>
<td>265</td>
<td>25 (9.4)</td>
<td>12.0</td>
<td>1.37 0.008</td>
<td>0 -</td>
<td>0 -</td>
</tr>
</tbody>
</table>

*: Five survived mothers had resorption sites only
**: Derived from 18 mothers
*: Significant at P<0.01

#: Code for type and site of abnormalities

CF: Club foot
CP: Cleft palate
DD: Digital defect
MG: Micrognathia
HD: Hematoma at digits
HF: Hematoma on face
Table 2B  Frequency of digital abnormalities classified according to their site

<table>
<thead>
<tr>
<th>Group</th>
<th>Forelimb</th>
<th>Hind Limb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>TB-500</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>TB-600</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>TSS-600</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

In all the treated groups the average body weight of the live fetuses was significantly decreased as compared with that of the control group.

C. Caffeine experiment

Caffeine (solubility: 1g/46 ml) suspended in 0.5% CMC suspension was administered at the two levels of dosage shown in Table 3A.

In both treated groups, the mothers showed dyspnea and slight convulsion for several minutes after treatment. About 30% of the mothers in the group CF-250 were found dead in the next morning. Fetuses with skeletal malformations and/or subcutaneous hematomas were found in both treated groups. Between these two groups a dose effect response is shown with respect to both teratogenicity and occurrence of subcutaneous hematoma.

Like in other experiments, the hematoma was found more frequently at the upper and/or lower jaw than in the digital regions. As for the sequence of incidence of various types of malformations, cleft palate was the first and micrognathia the second.

The frequency of digital abnormalities classified according to their site is shown in Table 3B.

Similarly to the results in Tables 1B and 2B, a left side preponderance is found in the group CF-250.

Mortality of the offspring was significantly higher in the group CF-250 than that of the control group.

Average body weight of the fetuses was significantly decreased in both treated groups as compared with that of the control group.

The above mentioned results indicate that a single injection of all four compounds, which are methylated xanthines, are teratogenic as well as causing hematomas when administered at the toxic dosage.
Table 3A  Effects of administration of caffeine (CF) given on gestational day 12 on mouse embryo

<table>
<thead>
<tr>
<th>Group (mg/kg)</th>
<th>No. of mothers treated</th>
<th>No. of maternal death</th>
<th>Total implants</th>
<th>No. of resorptions out of total implants (%)</th>
<th>Average litter size</th>
<th>Average body weight ± Sx</th>
<th>No. of malformed and their type* (% of total live)</th>
<th>No. with subcutaneous hematoma and their site* (% of total live)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF-200</td>
<td>20</td>
<td>0</td>
<td>273</td>
<td>22 (8.1)</td>
<td>12.6</td>
<td>1.27 ± 0.007</td>
<td>45* (17.9)</td>
<td>AG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CF 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CP 41</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>MG 10</td>
<td></td>
</tr>
<tr>
<td>CF-250</td>
<td>27+</td>
<td>8</td>
<td>231++</td>
<td>75* (32.5)</td>
<td>9.2</td>
<td>1.23 ± 0.010</td>
<td>108* (69.2)</td>
<td>CP 106</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DD 23</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MG 29</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>0</td>
<td>265</td>
<td>25 (9.4)</td>
<td>12.0</td>
<td>1.37 ± 0.008</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

+: Two survived mothers had resorption sites only
**: Derived from 17 mothers
*: Significant at P<0.01

#: Code for type and site of abnormalities
AG: Agnathia
MG: Micrognathia
CF: Club foot
HD: Hematoma at digits
CP: Cleft palate
HF: Hematoma on face
DD: Digital defect
The type of malformations was mainly skeletal defects such as cleft palate, digital defects and micrognathia. Considering the type of malformations, the effect of theophylline or theobromine sodium salicylate seems to be more similar to that of caffeine than that of theobromine.

On the other hand, Georges and Denef ('68) reported that the limb abnormalities induced by administration of theophylline related compounds were shown with a peculiar high frequency in the left posterior limb. Also, in our experiments, the digital defects occasionally accompanied by the hematoma occurred more frequently on the left fore-or hind limb than those on the right limbs in most of the treated groups. The causal mechanism of the above mentioned side preponderance of teratogenic effects awaits further study.

Summary

Theophylline (175, 200 and 225 mg/kg), theobromine (500 and 600 mg/kg), theobromine sodium salicylate (600 mg/kg) and caffeine (200 and 250 mg/kg) were injected intraperitoneally to the ICR-JCL mice once on the day 12 of gestation and their fetuses were examined for their abnormalities on the day 18 of gestation.

Main findings are as follows:
1. All of these compounds induced significantly high incidences of the fetuses with malformations and/or subcutaneous hematomas mostly in the adjacent region.
2. The type of malformations in all the treated groups was mainly skeletal defects such as cleft palate, digital defects and micrognathia. There was some difference of their frequencies among the kinds of compound.
3. There may exist a tendency that the digital defects and hematomas occur more frequently on the left fore-and hind limb
than those on the right limbs in most of the treated groups.

4. In the groups treated with larger dosage of theophylline, theobromine and caffeine, a lethal effect on the fetuses was shown.

5. Body weight of the live fetuses was significantly decreased in all the treated groups except for the treated with the lowest dosage of theophylline.

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References


