Dilatation of Cerebral Ventricles of Rat Offsprings Induced by 6-Mercaptopurine Administration to Dams

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Microcephaly and dilatation of cerebral ventricles not accompanied with other gross malformations were induced in rat offsprings by an intraperitoneal administration of 6-mercaptopurine in a dose of 20 mg/kg on the eleventh day of gestation of dams.

Microcephaly and dilatation of cerebral ventricles were often found in cases with formiminotransferase deficiency syndrome\(^1\) or with probably cyclohydrolase deficiency syndrome.\(^1\)

We suggested that microcephaly and dilatation of cerebral ventricles in those cases might have resulted from an impaired purine biosynthesis of the brain in fetal life due to a defective activity in tetrahydrofolate dependent enzymes such as formiminotransferase or probably cyclohydrolase.

The present investigation was undertaken to afford an experimental evidence in supporting of our speculation above quoted, and revealed that microcephaly and dilatation of cerebral ventricles not accompanied with other gross malformations could be induced in rat offsprings when 6-mercaptopurine was given to the dams intraperitoneally in a dose of 20 mg/kg on the eleventh day of gestation.

**Experiments**

The present experiments were conducted with female rats of the Wistar strain supplied by the Institute for Tuberculosis, Leprosy and Cancer Research, Tohoku University. The animals were maintained on a standard diet purchased from Oriental Co. Tokyo, Japan.

The onset of pregnancy was determined by examining the vaginal content of sperm, and the morning of massive sperm findings in the vagina was considered as day zero of gestation.

Fetalized animals were divided into four groups: Group I included one rat without any medication and was used as a control; Group II, consisting of 6

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rats, were given 6 MP (6-mercaptopurine) in a dose of 45 mg/kg of bodyweight; Group III, consisting of 5 rats, receiving 6 MP, 30 mg/kg of bodyweight, and Group IV, consisting of 6 rats, receiving 6 MP, 20 mg/kg of bodyweight.

An administration of 6 MP was done intraperitoneally only on the 11th day of gestation.

Immediately after birth each of the offsprings was observed for the occurrence of gross malformations such as cleft palate, curved short tail and adactylia.

Methods for evaluation of dilatation of cerebral ventricles and microcephaly were as follows: Each of the offsprings was weighed and then kept in a 10% formalin solution for a period of one month.

A slice, 1 mm in thickness, of frontal section of the head at the site halfway between the eye and ear was made and photographed. By using the photographs of 4 to 5 times magnifications, following measurements were done: A (cm), a largest distance between most lateral contours of the cellae mediae; and B (cm), an internal transverse diameter of the skull (cf. Fig. 1).

Fig. 1. Schematic illustration of measurement of an index for evaluating dilatation of the cerebral ventricle in a frontal section of the head of rats.
A: Largest distance between most lateral contours of the cellae mediae.
B: Internal transverse diameter of the skull.

The ratio of A/B was used as an index for evaluating a grade of dilatation of cerebral ventricles. The ratio of B/C served as an index for estimating a grade of microcephaly, where C (cm) represented a circumference of the chest of offsprings.

RESULTS AND DISCUSSION

Incidence in gross malformations of offsprings from dams with an administration of various doses of 6 MP

As was shown in Table I, it was found that all of malformations such as cleft
Dilatation of Cerebral Ventricles by 6MP

Table 1. Incidence in malformation of rat offsprings from dams with an intraperitoneal administration of 6 mercaptopurine on the eleventh day of gestation

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of 6MP (mg per kg)</th>
<th>Mothers (No.)</th>
<th>Body-weight (average) (g)</th>
<th>Ventricular dilatation</th>
<th>Cleft palate</th>
<th>Curved short tail</th>
<th>Adactylia</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>45</td>
<td>6</td>
<td>29</td>
<td>2.0</td>
<td>29 (100%)</td>
<td>29 (100%)</td>
<td>29 (100%)</td>
</tr>
<tr>
<td>II</td>
<td>30</td>
<td>5</td>
<td>52</td>
<td>2.2</td>
<td>52 (100%)</td>
<td>41 (78%)</td>
<td>52 (100%)</td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>6</td>
<td>58</td>
<td>4.4</td>
<td>58 (100%)</td>
<td>0</td>
<td>7 (12%)</td>
</tr>
<tr>
<td>IV</td>
<td>None</td>
<td>1</td>
<td>11</td>
<td>5.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2. Rat offsprings from dams to which 6MP was given intraperitoneally on the 11th day of gestation.
A: Control.
B: Rat offspring from a dam receiving 6MP, 20 mg/kg.
C & D: Rat offsprings from dams receiving 6MP, 30 mg/kg.
E: Rat offspring from a dam receiving 6MP, 45 mg/kg.

Development of 'microcephaly' and 'dilatation of cerebral ventricles' of offsprings from dams with an administration of 6 MP in a dose of 20 mg/kg

As mentioned above, only 7 out of 58 offsprings from dams receiving 6MP
### Table 2. Indices for evaluating microcephaly and dilatation of cerebral ventricles of rat offsprings from dams with or without an intraperitoneal injection of 6 MP, 20 mg/kg, on the eleventh day of gestation

<table>
<thead>
<tr>
<th>Group</th>
<th>Dams (No.)</th>
<th>C (Chest circumference, cm)</th>
<th>B (Internal transverse diameter of the skull, cm)</th>
<th>B/C</th>
<th>A (Largest distance between most lateral contours of cellae mediae, cm)</th>
<th>A/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>11</td>
<td>4.3 (4.2-4.4)</td>
<td>0.88 (0.77-0.94)</td>
<td>0.20 ± 0.08</td>
<td>0.24 (0.21-0.27)</td>
<td>0.27 ± 0.05</td>
</tr>
<tr>
<td>(Control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>68</td>
<td>4.3 (4.2-4.5)</td>
<td>0.72 (0.64-0.85)</td>
<td>0.17 ± 0.01</td>
<td>0.30 (0.19-0.46)</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>(6MP, 20 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

in 20 mg/kg (Group III), showed the curved short tail as a gross malformation. The cleft palate and adactylia were not found in any of offsprings of Group III (cf. Table 1). However, as was shown in Table 2 and Fig. 3, the ratio of A/B was found to be 0.27±0.05 and 0.37±0.03 in Groups IV and III respectively, indicating that cerebral ventricles were dilatated in offsprings of Group III as compared with those in Group IV.

Furthermore the ratio of B/C was found to be 0.20±0.08 and 0.17±0.01 in Groups IV and III, respectively, indicating that there was a tendency toward microcephaly in offsprings of Group III (cf. Table 2).

Wilson and Warkany\(^2\) described that a teratogenic effect of 6 MP was only seen in rats when the female animals were treated with 6 MP on either 11th day or/and 12th day of gestation. The abnormalities of rat fetus included encephalocele, polydactylia, cleft palate, curved short tail, ectodactylia, adactylia and retarded limbs.

Our results of the present experimentation showed a good accordance with those of Wilson and Warkany so far as 6 MP was given in a dose ranging from 30 mg/kg to 45 mg/kg intraperitoneally on the 11th day of gestation of rats.

On the other hand, when 6MP was given intraperitoneally to rats in a dose of 20 mg/kg on the 11th day of gestation, only microcephaly and dilatation of cerebral ventricles of rat offsprings were induced without accompanying any other gross malformations such as cleft palate and adactylia. This finding revealed that the dose of 20 mg/kg of 6 MP was sufficient for causing an impairment in purine biosynthesis in the brain in the early stage of fetal life of rats, but it was not enough to cause other gross malformations.
Mental retardation, microcephaly, brain atrophy and dilatation of cerebral ventricles were found often in both the formiminotransferase deficiency and probable cyclohydrolase deficiency syndromes. We are of the opinion that the brain atrophy and dilatation of cerebral ventricles in those syndromes may result from an impaired purine biosynthesis of the brain in the fetal life due to a formiminotransferase or cyclohydrolase deficiency.

The results of our present experimentation seems to afford an evidence supporting our speculation above mentioned.
References
