Side Preponderant Forelimb Defects of Mouse Fetuses Induced by Maternal Treatment with Adenine

By

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It has been demonstrated that adenine, a normal purine metabolite, is teratogenic in mice (Fujii, '70; Fujii and Nishimura, '70) and rat (Fujii and Nishimura, in press) if administered to the pregnant female at a high dose during the period of major organogenesis of the embryo.

In the previous study (Fujii, '70), it was slightly suggested that forelimb defects by adenine in mice on day 10 of gestation occur in a side preponderant manner. The present paper deals mainly with the peculiar asymmetric phenomenon shown in the occurrence of forelimb defects, which were confirmed in our subsequent study.

Materials and Methods

ICR-JCL strain mice were purchased at 3 weeks of age from Japan CLEA Co. (Takatsu ki, Osaka) and maintained until 9 weeks of age in the animal room of N.M.B. Research Laboratories (Okazaki). Then, each virgin female was placed overnight (17 hours) with an adult male of the same strain. Copulation was established by the presence of the vaginal plug next morning, and this day was designated as day 1 of gestation. Animals were kept on the solid chow (CA-1, Japan CLEA Co.) and fresh water ad libitum. Experiments were carried out in the animal room where the temperature (22±1°C) and humidity (55±5%) were maintained at constant levels. The pregnant mice were divided into three groups: two experimental

1) Adenine manufactured by Sigma Chemical Company (St. Louis, Mo., U.S.A.) was obtained through Katayama Chemical Company (Osaka, Japan).
and a control. The experimental groups were injected once intra-
peritoneally on day 10 of gestation with 200 (low dose group) or
250 mg/kg (high dose group) of adenine suspended in 0.5% CMC
(Carboxymethyl Cellulose) solution. The concentration was adjusted
so that the volume of a dose was 10 ml/kg. The control group re-
ceived the same volume of the CMC vehicle in the same way as the
experimental groups. All dams were sacrificed on day 19 of gestation.
The implantation sites and the signs of death such as resorption or
maceration of the fetuses were checked *in situ* and all living fetuses
were removed from the uterus, weighed and examined for external
deformities including those in the oral cavity.

Results

All dams, in both experimental groups, showed a slight twitching
of abdominal muscles and decreased bodily movement for several
minutes after receiving the injection.

The autopsy data are summarized in table 1.

<table>
<thead>
<tr>
<th>Group (mg/kg)</th>
<th>No. of mothers treated</th>
<th>No. of whole litter resorption</th>
<th>Total implants</th>
<th>No. of resorbed or dead fetuses* (%)</th>
<th>Average litter size</th>
<th>Average body weight ±SX (g)</th>
<th>No. of externally malformed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>18</td>
<td>1</td>
<td>234</td>
<td>69** (29.5)</td>
<td>9.7</td>
<td>1.23** 0.010</td>
<td>20** (12.1)</td>
</tr>
<tr>
<td>250</td>
<td>21</td>
<td>8</td>
<td>190</td>
<td>80** (42.1)</td>
<td>8.5</td>
<td>1.18** 0.013</td>
<td>14** (12.7)</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>0</td>
<td>226</td>
<td>16 (7.1)</td>
<td>13.1</td>
<td>1.36 0.008</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>

* Number from cases with whole litter resorption is excluded.
** Significant at P<0.01 when compared with control.

Embryocidal, growth suppressing and teratogenic effects were
shown in both experimental groups. A dose effect relationship is
indicated with respect to lethality and growth suppression.

The detailed results on the external malformations are shown in
table 2.

There were 11 cases of forelimb defects when both experimental
groups were combined. As is shown in table 3, it is noteworthy that
most cases (nine out of 11) are left-sided and its occurrence is
significant at P<0.05 compared as the right-sided defects. Figure 1
Table 2. Classified external malformations caused by maternal adenine injection (ip) on day 10 of gestation in mice.

<table>
<thead>
<tr>
<th>Group (mg/kg)</th>
<th>No. of live fetuses</th>
<th>No. of fetuses with external malformation (%)</th>
<th>Encephalocele</th>
<th>Exencephaly with open eyelid</th>
<th>Cleft palate with/without unilateral cleft lip</th>
<th>Forelimb defect</th>
<th>Tail defect (shortened, bent or absent)</th>
<th>Imperforate anus</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>165</td>
<td></td>
<td>4 (2.4)</td>
<td>0</td>
<td>10 (6.1)</td>
<td>6 (3.6)</td>
<td>8 (4.8)</td>
<td>0</td>
</tr>
<tr>
<td>250</td>
<td>110</td>
<td></td>
<td>0</td>
<td>0</td>
<td>5 (4.5)</td>
<td>5 (4.5)</td>
<td>8 (7.3)</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Control</td>
<td>210</td>
<td></td>
<td>0</td>
<td>1 (0.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Side preponderance of various malformations in the forelimbs caused by maternal adenine injection (ip) in mice.

<table>
<thead>
<tr>
<th>Type of malformation</th>
<th>No. of fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left amelia</td>
<td>4</td>
</tr>
<tr>
<td>Left hemimelia</td>
<td>3</td>
</tr>
<tr>
<td>Left hemimelia and right adactyly</td>
<td>1</td>
</tr>
<tr>
<td>Left adactyly</td>
<td>1</td>
</tr>
<tr>
<td>Left brachydactyly</td>
<td>1</td>
</tr>
<tr>
<td>Right syndactyly</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
</tr>
</tbody>
</table>

1. Left side preponderance is significant at P<0.05.

shows the typical cases with such a side preponderance.

The forelimb defects were often accompanied with other types of malformations such as cleft palate, cleft lip and tail defects.

Discussion

Grüneberg ('63) describes asymmetric occurrence of various limb anomalies in mice with mutant genes. It can be postulated that a right-sided occurrence is more common in these mutant defects. As for a side preponderance of forelimb defects of mice induced by prenatal environmental influences, Layton and Hallesy ('65) and Hallesy and Layton ('67) reported that oral administration of very high doses of carbonic anhydrase inhibitors such as dichlorphenamide or acetazolamide to pregnant rats produced postaxial defects of the right forelimb in most cases. Moreover, Wilson et al. ('68) reported that acetazolamide and ethoxyzolamide, another carbonic anhydrase inhibitor, incorporated into the diet of pregnant rats caused localized malformations typically affecting the distal postaxial part of the right
Fig. 1. Left-sided forelimb defects of various grades in the mouse fetuses caused by maternal injection of adenine (ip) on day 10 of gestation.
forelimb. The similar side preponderance of forelimb defects was also found in ICR mice treated with acetazolamide (Suzuki and Takano, '69). On the other hand, Georges and Denef ('68) reported that the abnormalities in the left posterior limb were induced with a high frequency when aminophylline was injected subcutaneously to pregnant rats from day 1 to 17 of gestation. Fujii and Nishimura ('69) also found that some methylated xanthines such as caffeine, theophylline and theobromine administered to pregnant mice on day 13 of gestation induced digital defects of the fetuses with a higher frequency on the left side than on the right. Furthermore, in thalidomide experiments in the rabbit, it was reported that forelimb defects were more numerous on the left side and on average more severe on this side (Erfurth, '65; Pearn and Vickers, '66; Vickers, '67; Schumacher et al., '68; Vickers and Wrba, '70).

No clearcut explanation for mechanism of such sided impairment has been established. However, one of the possibilities may be introduced from a known fact that the right side of mouse and rat embryos at midpregnancy faces the placenta (Snell and Stevens, '66; Nicholas, '63; Wilk, '69). We examined separately 10-day embryos in utero of five female mice of ICR-JCL strain and confirmed that the right side, that is, the right limbs of all of those embryos were toward the placentas, the left being toward the antimesometrial portion of the membranes. It may be speculated that the transport of adenine or its metabolites takes place via the amniotic sac to the surface of embryos and both sides of the body are exposed to the compounds at different concentrations due to the above mentioned specific embryonic position.

Summary

Adenine was injected intraperitoneally to pregnant ICR-JCL mice on day 10 of gestation at a dosage of 200 or 250 mg/kg. The fetuses were examined externally on day 19 of gestation. Embryonic death, growth suppression and malformations such as encephalocele on forehead, cleft palate, cleft lip, limb defects and tail defects were induced. It is noteworthy that left-sided malformations were found in nine out of 11 fetuses with forelimb defects.

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**Literature cited**


