Transmission of Tumors and Malformations to the Next Generation of Mice Subsequent to Urethan Treatment

Taisei Nomura

First Department of Surgery, Osaka University Medical School, Dōjima-hamadori 3-1-2, Fukushima-ku, Osaka 553, Japan

Urethan possesses 3 types of transplacental toxicity. When urethan was given during pregnancy, it caused embryonic deaths, malformation, and neoplasm in the offspring (3, 4). The author reported an analytical study of the effects of urethan on the developing mouse embryo in relation to these transplacental toxicities (5-7), utilizing urethan's unique characteristics of fast action and complete placental penetration (8). During these experiments, tumors were detected in the 2nd generation of descendants, obtained accidentally from the offspring receiving urethan on Day 17 of gestation. Consequently, an analytical study on the transmission of tumors to the next generation has been carried out from 1967 in the manner shown in Chart 1.

ICR/Jc1 male and female mice (Japan Central Laboratory for Experimental Animals, Tokyo, Japan), 9 to 10 weeks old and weighing 25 to 30 g, were used. Male and female mice from one litter were mated in the evening, and on the next morning a vaginal plug was checked to determine Day 1 of gestation (7). Pregnant mice were divided into 3 groups (Chart 1, Groups A to C). Group A (controls) received a single s.c. injection of distilled water on Day 17. Group B received a single s.c. injection of urethan (1.0 mg/g body weight) on Day 17, and their offspring were foster-nursed by nontreated, lactating (Group C) mothers immediately after birth, in order to exclude any milk factor. Group C mice were nontreated, and their offspring were foster-nursed by the Day 17 urethan-treated mothers (Group B mothers) immediately after birth. These male and female offspring (A0, B0, and C0) were mated with each other 8 to 10 weeks after birth, as shown in Chart 1, and the 2nd generation of descendants (A1, B1, A1B1, and C1) was obtained. Also, a 3rd generation of descendants (B2) was obtained from the 2nd generation (B1) of Group B. These descendants were maintained on mouse diet CA-1 (CLEA Japan, Tokyo, Japan) and water. [Components of mouse diet CA-1 have been given in a previous paper (6).] They were sacrificed 25 weeks after birth, and gross pathological lesions were examined especially for tumors and malformations. The lungs were examined again after fixation in 20% neutral formaldehyde solution, and small tumors were determined histologically by serial sections. These procedures were repeated 4 times from 1968 to 1971. Results are summarized in Table 1. Group B offspring (B0) developed significantly more tumors than did the control group offspring (A0); and the next descendants (B1) obtained from these offspring also developed a significant number of tumors. It may be suggested from these findings that germ cells in the Day 17 fetus were exposed to urethan, and tumors were transmitted to the next generation, because when urethan was given to pregnant mice at any stage of gestation, it was uniformly distributed in all organs of both mothers and their fetuses, and its toxicity was limited to a rather short period of 24 hr (8). An increased incidence of tumors was also observed in Group C offspring (C0) and their descendants (C1). Small amounts of urethan may reach the newborn via mother's milk (5, 8). However, tumors were not observed in the 2nd generation of descendants (A1B1) obtained from the male offspring exposed to urethan on Day 17 and the female offspring of the control group. There was also no difference in tumor incidence between the 3rd generation descendants (B2) and controls (A1). Although the same procedure was conducted regarding lung and tail anomalies that were induced when 1.5 mg of urethan per g body weight were given on Day 9 (5, 6), these malformations were not observed in 25

Chart 1. Diagram of experimental procedure. Regarding pregnant mice of Groups A, B, and C, a vaginal plug was recognized on the same day. Details are given in the text.

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Letter to the Editor

Tumor incidence in the 1st, 2nd, and 3rd offspring generations of mice receiving urethan during pregnancy

Experimental procedures are given in the text and in Chart 1.

Table 1

<table>
<thead>
<tr>
<th>Experimental groups a</th>
<th>Live births</th>
<th>Tumor-bearing mice</th>
<th>Lung tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. no.</td>
<td>Total no.</td>
<td>Incidence (%)</td>
</tr>
<tr>
<td>A0</td>
<td>10.4 ± 1.0 d (7)</td>
<td>73</td>
<td>5/72 (6.9)</td>
</tr>
<tr>
<td>B0</td>
<td>10.2 ± 1.0 (6)</td>
<td>61</td>
<td>42/52 (80.8)</td>
</tr>
<tr>
<td>C0</td>
<td>11.7 ± 0.2 (7)</td>
<td>82</td>
<td>13/70 (18.6)</td>
</tr>
<tr>
<td>A1</td>
<td>10.4 ± 0.5 (25)</td>
<td>260</td>
<td>14/237 (5.9)</td>
</tr>
<tr>
<td>B1</td>
<td>9.4 ± 0.1 (13)</td>
<td>122</td>
<td>18/112 (16.1)</td>
</tr>
<tr>
<td>C1</td>
<td>10.6 ± 0.5 (26)</td>
<td>275</td>
<td>21/197 (10.7)</td>
</tr>
<tr>
<td>A1 B1</td>
<td>10.4 ± 0.6 (8)</td>
<td>83</td>
<td>4/82 (4.9)</td>
</tr>
<tr>
<td>B1</td>
<td>12.2 ± 0.3 (13)</td>
<td>159</td>
<td>7/156 (4.5)</td>
</tr>
</tbody>
</table>

a Controls (A0 and A1) were sacrificed 32 weeks after birth.
b The t test was applied after testing variance ratio. If the variance ratio was over F value at 5%, the t test was applied with approximation of Cochran-Cox. All statistical analyses were performed as follows: B0 versus A0; B1, C1, A1, B1, and B2 versus A1.
c LY, lymphoma (node type); HE, hepatoma; OV, ovarian cystadenoma.
d Mean ± S.E.
e Numbers in parentheses, number of mice that delivered live offspring.
f NS, not significant.

Table 2

Incidence of tumors and malformations in the offspring of urethan-exposed male and female mice

Details of the experimental procedure are given in the text.

<table>
<thead>
<tr>
<th>Treatment to parents</th>
<th>Live births</th>
<th>Tumor-bearing mice</th>
<th>Lung tumors a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. no.</td>
<td>Total no.</td>
<td>Incidence (%)</td>
</tr>
<tr>
<td>Urethan (1.5)</td>
<td>10.7 ± 0.3 c (68) d</td>
<td>730</td>
<td>75/621 (12.1)</td>
</tr>
<tr>
<td>Untreated</td>
<td>9.2 ± 0.3 f (65)</td>
<td>597</td>
<td>75/466 (16.1)</td>
</tr>
<tr>
<td>Urethan (1.5)</td>
<td>10.3 ± 0.2 (94)</td>
<td>968</td>
<td>115/772 (14.9)</td>
</tr>
<tr>
<td>Untreated</td>
<td>11.1 ± 0.1 (76)</td>
<td>844</td>
<td>56/809 (6.9)</td>
</tr>
</tbody>
</table>

a Mice with pneumonia were excluded.
b LY, lymphoma (T, thymic type; N, node type; S, splenic type; M, mixed type); OV, ovarian cystadenoma R, renal tumor; HE, hepatoma LI, lipoma; TH, thyroid tumor; K, kinky tail; A, atresia hymenalis; HY, hydrocephalus.
c Mean ± S.E.
d Numbers in parentheses, number of mice that delivered live offspring.
e Application of t test yielded p value of 0.001.
f Controls were sacrificed 32 to 50 weeks after birth.

descendants obtained from the malformation-bearing male and female offspring.

In order to analyze effects of urethan on germ cells, measurement has been made of dominant lethality, teratogenicity, and tumorigenicity in the offspring of urethan-exposed males and females. When male mice (9 weeks old) received a single s.c. injection of urethan (1.5 mg/g body weight), and were subsequently mated with nontreated females (9 weeks old) at 1- to 10-week intervals between injection and conception, 168 of 177 females with vaginal plugs (94.9%) became pregnant. Eighty-one of them were sacrificed on Day 19, and CL, implants, PRE, ED, late deaths, living fetuses,
and malformations of the external appearance were examined. The dominant lethality (PRE + ED/CL) (2, 9) was 24 of 122 (19.7%) at 3-week intervals (spermatid stage), and was significantly different from controls [48 of 641 (7.5%), p < 0.001] and other experimental stages [1-, 2-, and 4- to 10-week intervals, 93 of 931 (10.0%), p < 0.002]. Malformed fetuses were also observed in 18 of 897 living fetuses (2.0%, p = 0.05). When female mice received urethan, 1.5 mg/g, and were subsequently mated with nontreated males at 1- to 10-week intervals, 177 of 199 females with vaginal plugs (88.9%) reached pregnancy. The average number of CL (12.3 ± 0.3) was significantly lower than controls (13.3 ± 0.2, p < 0.01), and the group of urethan-exposed males (13.0 ± 0.1, p < 0.02). PRE + ED/CL was higher at 2- to 3-week intervals (39 of 270, 14.4%) than controls (p < 0.002) and other experimental stages [1- and 4- to 10-week intervals, 77 of 741 (10.4%), p = 0.07]. Malformed fetuses were also observed in significantly higher incidence [27 of 856 (3.2%), p < 0.01] than controls (4 of 551, 0.7%). Malformations consisted of open eyelids, kinked tails, cleft palates, and dwarfism. There was no significant difference in the incidence of late deaths among the groups of urethan-exposed males (39 of 1053, 3.7%), of females (38 of 1011, 3.8%), and of controls (18 of 641, 2.8%). Offspring of 68 urethan-exposed males and those of 65 urethan-exposed females were sacrificed 32 weeks after birth, and offspring of 94 females treated in the same way with 1.0 mg of urethan per g body weight were also sacrificed. The incidence of tumors and malformations is summarized in Table 2. Tumors were induced in significantly higher numbers in the offspring of urethan-exposed males and females than in the offspring of controls. Furthermore, tumors and malformations were observed in higher incidence in the offspring of urethan-exposed females than in those of urethan-exposed males. It may be suggested from these findings that urethan damages not only the chromosomes but also the other components of oocytes, although dominant lethality in the offspring of urethan-exposed males indicates the induction of chromosome aberrations (1). Further genetic analysis remains to be done in future research.

Urethan-exposed mother mice developed uterine hemangiomas (13 of 105, 12.4%).

REFERENCES

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