Urethan and Leukemogenesis in Mice*

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Urethan suppresses hemopoietic activity in experimental animals (18) and is "radiomimetic" in other respects. It induces pulmonary tumors in rodents (10, 11); when administered orally to mice, papillomas of the forestomach may appear (1). Urethan initiates skin carcinogenesis in mice, although when administered alone skin tumors do not appear (14). Hemangiomas of the mouse liver have been induced by this chemical (6).

The objective of this investigation was to test the influence of this radiomimetic agent on mouse leukemogenesis. X-rays are independently leukemogenic, or may act either additionally or synergistically with other leukemogenic agents (e.g., methylcholanthrene, estrogenic hormone) to augment the induction of mouse leukemia (3, 9). If urethan by itself is not leukemogenic, but augments or promotes leukemogenesis, then it could be classified as a "co-leukemogen," following the terminology of Berenblum in designating agents as "co-carcinogens" (2). Urethan was administered as follows: (a) independently, (b) together with x-rays, (c) with estrogenic hormone, (d) with both of these agents, and (e) with methylcholanthrene, to test its influence on leukemogenesis in pure strain C57BL, BALB/c, and DBA/2 mice, and C57BL-C3H F1 hybrids. All these stocks are considered to be "low-leukemia" from the standpoint of spontaneous occurrence (Chart 9).

In addition, experiments are being reported involving the effect on mouse leukemogenesis of shielding bone marrow or thymus during x-irradiation under urethan anesthesia. In preliminary experiments it had been found (7) that thigh-shielding of C57BL mice under urethan anesthesia did not protect against the induction of leukemia, this observation leading to the present studies on augmentation of mouse leukemogenesis by urethan. Either thigh-shielding or thymus-shielding under barbiturate anesthesia (5) is protective.

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EXPERIMENTAL

X-rays combined with urethan: leukemogenic effects.—Urethan was administered together with a dose of x-rays of low leukemogenic activity to the following groups of C57BL mice (Chart 1), beginning at 6 weeks of age. C57BL mice are susceptible to the leukemogenic action of x-rays (4).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage</th>
<th>Age at Death (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-R(80 r)</td>
<td>18%</td>
<td>55.5%</td>
</tr>
<tr>
<td>X-R(40 r) + Urethan</td>
<td>35%</td>
<td>9.6%</td>
</tr>
<tr>
<td>Urethan Only</td>
<td>45%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Chart 1.—Effect of urethan on induction of leukemia by x-rays in intact (male and female) C57BL mice. Treatment with urethan and x-rays begun at 6 weeks of age.

1. 40 r of x-rays1 whole-body under urethan anesthesia every 4th day, for 11 times. The dose of urethan (ethyl carbamate) was 1 mg/gm of body weight, administered intraperitoneally in 10 per cent aqueous solution.
2. Same x-ray treatment—no urethan.
3. Urethan only—unirradiated.

1 Physical factors: 140 kvP, 5 ma., 2.0 mm. Al. added filter, 30 cm. target mouse distance. Output, 58.8 r/min.
4. 80 r of x-rays whole-body every 4th day 11 times—no urethan.

In a similar manner, the effect of urethan on irradiation-induced leukemogenesis was tested with BALB/c and DAB/2 mice (Charts 2 and 3). For each strain, groups of mice given the indicated treatments (beginning at 6 weeks of age) were observed. Both of these strains were known to be susceptible to the induction of leukemia by x-rays (8).

1. 90 r of x-rays whole-body under urethan anesthesia every 4th day, for 4 times.
2. Same treatment with x-rays—no urethan.
3. Urethan only—unirradiated.

<table>
<thead>
<tr>
<th>Whole Body</th>
<th>Urethan</th>
<th>Whole Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 r x 4</td>
<td>51 28.9%</td>
<td>25 8.0%</td>
</tr>
<tr>
<td>+ Urethan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urethan</td>
<td>0 26.0%</td>
<td></td>
</tr>
</tbody>
</table>

**Chart 2.**—Effect of urethan on induction of leukemia by x-rays in intact (male and female) BALB/c mice. Treatment begun at 6 weeks of age.

In all three strains, although urethan per se was not leukemogenic, it significantly augmented the induction of leukemia by x-rays (Charts 1–3).

**Estrogenic hormone combined with urethan: leukemogenic effects.**—To determine whether urethan might act synergistically with estrogenic hormone in inducing leukemia, the following groups of C57BL mice were studied (Chart 4).

1. 25 Micrograms of estradiol dipropionate in oil (CIBA) weekly, given subcutaneously.
2. Same treatment plus urethan every 4th day, 11 times.
3. Urethan only, no estradiol.

In this strain, compared with others, estrogenic hormone proved to be only mildly leukemogenic; urethan alone induced no leukemia. In mice receiving both agents, the incidence of leukemia was almost 60 per cent, a synergistic effect (Chart 4).

**Estrogenic hormone combined with both urethan and x-rays: leukemogenic effects.**—The supplemental effect of urethan on the combined (additive) leukemogenic action of x-rays and estrogenic hormone was studied in C57BL-C3H F1 hybrids (Chart 5). The incidence of leukemia in untreated controls is indicated in Chart 9. The following groups of F1 hybrid mice were studied:

1. 40 r of x-rays whole-body every 4th day, 11 times—no anesthesia.
2. Same treatment with x-rays under urethan anesthesia. One thigh was shielded, a treatment which under nembutal anesthesia protects against leukemogenesis.
3. 25 µg. of estradiol dipropionate weekly.
4. Same treatment with estradiol dipropionate plus 40 r of x-rays to the whole body every 4th day, 11 times.
5. Estradiol dipropionate, plus urethan anesthesia 11 times at 4-day intervals when 40 r of x-rays were given with the left thigh shielded.

Urethan supplemented the combined leukemogenic effects of x-rays and estrogenic hormone (Chart 4). Whole-body exposure of 90 r at 4-day intervals for 4 times was not as active in inducing leukemia as 40 r X 4 with one thigh shielded under urethan anesthesia. Combined treatment with 40 r X 4 (whole-body, no urethan) and estrogenic hormone yielded 48 per cent leukemia, whereas the addition of urethan resulted in an earlier appearance of leukemia and a higher incidence (60 per cent), even though thigh shielding was employed during x-radiation.

A similar experiment was conducted on C57BL mice (Chart 6) with a larger dose of x-rays, 40 r X 11, rather than 40 r X 4. The incidence of leukemia in mice that were thigh-shielded under urethan anesthesia was 57.5 per cent. If mice were, in addition, estrogen-treated, the incidence was increased to 57.2 per cent with earlier onset. If thigh shielded mice were irradiated under amytal (rather
than urethan) anesthesia and treated with estrogen, the leukemia incidence was only 15.8 per cent (three out of nineteen), not significantly different from the incidence with estrogen only (Chart 5). Although the incidence of leukemia was 59.4 per cent in mice receiving estrogen plus urethan, the addition of urethan accelerated onset (Chart 6).

Influence of thigh and thymus shielding during x-radiation upon potentiation of leukemogenesis by urethan (Charts 6 and 7).—When C57BL mice were thigh-shielded during x-radiation under sodium amytal anesthesia, the leukemogenic effects of 80 r every 4 days X 11 were remarkably reduced. If the same treatment was given under urethan anesthesia, the incidence of leukemia was higher (39.1 compared with 18.2 per cent) and onset earlier (Chart 7). Although leukemogenesis was only moderately inhibited by thigh shielding under urethan anesthesia, thymus shielding was more effective (Chart 7).

The difference between thymus and thigh shielding is further demonstrated by the results illustrated in Chart 6. When mice were thigh-shielded under urethan anesthesia and given estrogenic hormone, the incidence of leukemia was 57.2 per cent. However, when the mice were thymus-shielded during x-radiation and similarly given urethan and estrogenic hormone, the leukemia incidence was only 26.1 per cent. Since this incidence is lower than when estrogenic hormone and urethan were given without irradiation, it is concluded that in C57BL mice extra-thymic irradiation inhibits estrogen-urethan-induced leukemogenesis.

Contrary to the results of Toch et al. (15), the incidence of leukemia in both thigh- and thymusshielded mice receiving estrogenic hormone was low when amytal anesthesia was employed during irradiation.

Effect of urethan on induction of leukemia by methylcholanthrene (Chart 8).—DBA/2 mice were given either urethan or skin paintings of methylcholanthrene in benzene (0.25 per cent solution) alone. Either eighteen or nine skin paintings were given, a different skin site being used for each of the paintings (3 times a week). In a third group for each dose of methylcholanthrene, urethan and methylcholanthrene treatments were combined. Onset of leukemia was significantly accelerated, and the total incidence was greater when urethan was given in addition to the methylcholanthrene. As in the other strains, urethan by itself did not induce leukemia.

Controls.—The incidence of leukemia in pure strain controls is shown in Chart 9. All pure strains have been inbred in this laboratory, the DBA/2’s since 1945 when the original breeder pair was obtained from the Jackson Memorial Laboratory. C57BL, CB (BALB/c without mammary tumor agent), and C3H breeding stocks were obtained in 1952 from Dr. John Bittner, University of Minnesota, and have since been maintained by brother-sister matings. The controls were all breeding stock from which the test mice were derived. Data on 110 C57BL-C3H F1 hybrids are shown in Chart 9.

DISCUSSION

Urethan remarkably augments the leukemogenic activity of x-rays, estrogenic hormone, and methylcholanthrene in mice, although by itself it does not seem to be leukemogenic in low-leukemia strains, at least in the doses which augment leukemogenesis. The influence of urethan on "viral-leukemogenesis" is not known. Presently, high-
leukemia C58 and AKR mice are being treated with urethan beginning at weaning age, to determine whether the onset of the disease is hastened, and low-leukemia mice are being treated with urethan prior to the administration of cell-free leukemic tissue extracts.

It is remarkable that an agent without demonstrable leukemogenic activity so strongly augments leukemogenesis induced by other agents. There are probably other "radiomimetic" agents which produce similar enhancing effects upon the induction of leukemia. The problem of leukemogenesis may be complicated by the promoting action of agents which might escape detection as leukemogens if tested independently.

**Chart 8.**—Effect of urethan on induction of leukemia by methylcholanthrene in DBA/2 male mice. Treatment was begun at 6 weeks of age.

**Chart 9.**—A. Incidence of spontaneous leukemia in untreated C57BL mice; B. Incidence of spontaneous leukemia in untreated BALB/c mice. C. Incidence of spontaneous leukemia in untreated DBA/2 male mice; D. Incidence of spontaneous leukemia in untreated C57BL-C8H F1 hybrids. These charts represent the observed occurrence of spontaneous leukemia in the untreated mice of the strains used for testing the leukemogenic action of urethan in combination with leukemogenic agents.
The fact that a single agent such as urethan enhances the leukemogenic action of either polycyclic hydrocarbons, estrogenic hormone, or ionizing radiations makes it seem likely that there is a stage in the leukemogenic process common to all, where a single agent may exert its influence.

In DBA/2 and BALB/c mice as few as four anesthetic doses of urethan added to a dose of x-rays of relatively low potency (4 × 90 r) increased the incidence of leukemia 3½ times and accelerated its onset (Charts 2 and 3). The minimum effective dose of urethan is being determined.

Certain strains of mice are relatively refractory to the leukemogenic action of methylcholanthrene, and in these the combined action of urethan and the polycyclic hydrocarbons is being tested. The influence of orotic acid (and similar agents), found by Rogers (12) to inhibit the carcinogenic action of urethan on the lung, is now being tested in this laboratory for possible antileukemogenic action.

Urethan to a considerable degree nullifies the protection afforded by thigh (marrow) shielding against x-ray-induced leukemogenesis (Chart 7). Estrogenic hormone has a similar effect (15). On the other hand, as in the case of estrogenic hormone (15), shielding of the thymus during x-radiation under urethan anesthesia inhibits the induction of leukemia (Charts 6 and 7). Experiments in progress demonstrate that thig shielding of C57BL mice does not protect against the lethal effects of x-rays if the mice are urethan-anesthetized.

SUMMARY

1. Urethan augmented the induction of leukemia in mice by x-rays, estrogenic hormone, or methylcholanthrene.

2. Urethan was not leukemogenic for low-leukemia strains of mice when administered alone in doses which remarkably augmented the leukemogenic activity of other agents.

3. The protective effects upon leukemia induction of shielding bone marrow were nullified if mice were anesthetized with urethan during x-radiation.

REFERENCES


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