Significance of Newborn Age and Dose of Urethan in Leukemogenesis

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Summary

Urethan alone has been known to be slightly leukemogenic for newborn mice and even less so for the adults. The studies were made to determine whether infant mice have the same susceptibility to leukemogenesis as newborns in relationship to the dose of urethan delivered.

Urethan was injected repeatedly at 3 dose levels into C57BL x C3H F1 mice starting when less than 24 hr old or at 7 days of age. The injections were given i.p. at 3-day intervals for a total of 6 times. The total dose of urethan delivered/gm body weight was 2.1, 3.0, or 4.2 mg.

The mice treated for the 1st time at newborn age developed leukemia of 7, 32, and 74% for 3 dose levels, respectively. However, the animals initially exposed to urethan when 7 days old showed a significantly lower incidence of 0, 7, and 38%. The positive linear dose-response relationship was found for both age groups between the total dose of urethan delivered and the incidence of leukemia.

The results demonstrated that 7-day-old mice are less susceptible than newborns to urethan leukemogenesis, which could be compensated by increasing the dose of urethan. Findings also convincingly confirmed the leukemogenicity of urethan for mice. These observations were correlated with previous findings and discussed in relation to current knowledge concerning the high susceptibility of newborn mice to urethan leukemogenesis.

Introduction

Urethan can affect and influence the development of tumors in various tissues of mice (25, 26), rats (27), and Syrian golden hamsters (29). The 1st observation regarding its role in leukemogenesis was made by Kirschbaum and his associates (13), who found that urethan potentiated leukemogenic effect of X-ray, estrogen, or methylcholanthrene in low-leukemia strains of mice. Alone, however, it slightly accelerated the onset of leukemia in high-leukemia strains (14). X-ray leukemogenesis was augmented by urethan only if it was given concurrently with or after but not before the X-ray treatment (2). Viral leukemogenesis was also potentiated by this agent (4, 18).

Urethan alone induced leukemia when administered only once to newborn Swiss (8, 24), C3Hf (17), CTM (6), or repeatedly to C57BL (7) mice. Also, multiple stomach tube instillations of urethan to 7- or 8-day-old B6AF1/J mice were highly leukemogenic (16). On the contrary, the adult mice are less susceptible to the leukemogenic action of urethan (25, 28).

The experiments reported here were performed to determine whether infant mice have the same susceptibility to leukemogenesis as newborns in relationship to the dose of urethan delivered.

Other neoplasms developed due to the urethan treatment at a later age and for clarity of this presentation are not reported here. In order to allow a between-group comparison and an evaluation of dose-response relationship regarding leukemogenesis, our observations terminated when the animals were 35 weeks of age.

Materials and Methods

MICE. The animals utilized were of F1 generation of C57BL female and C3H male. Both parental strains were raised in this laboratory by brother-to-sister breeding since 1961. They originally came from Dr. Tannenbaum's laboratory at Michael Reese Hospital. After conception females were allocated at random to different experimental groups. When the mice were born they received their treatments either when less than 24 hr of age or 7 days old. Animals were weaned at about 30 days of age, at which time they were numbered, recorded, and housed in plastic cages in sets of 10. Sanicel was used as the bedding. The mice were kept in a temperature-controlled laboratory at 78°F. From weaning they were fed Rockland diet and given water ad libitum.

Throughout the experiment, mice were weighed and inspected periodically. When an animal showed symptoms of leukemia or malignant lymphoma—superficial diaphragmatic breathing, pigeon chest with or without enlargement of superficial lymph nodes—and was in a moribund state it was killed.

Mice dying or sacrificed during the experiment were examined for neoplasms and other pathology. Specimens were taken from all thymuses and involved lymph nodes and the spleen, liver, kidneys, and lungs whether they were grossly involved or not. The tissues were fixed in 10% formalin, processed, and stained with hematoxylin and eosin.

URETHAN. White, crystalline urethan (ethyl carbamate), reagent-grade, was utilized. Solutions of 14, 10, or 7% concentration were always made in redistilled water shortly before use.

TREATMENT. Intraperitoneal route of application was utilized throughout all experiments reported here. Hamilton micro-syringes with 30-gauge needles were used in order to deliver a desired volume of a given urethan solution. This was always given according to the body weight at the time of injection. Concentrations were prepared in such a way that the desired amount of urethan per gm body weight was in 0.005 ml of solu-
Results

At the moment, the fingers were shifted toward the head, gripped during the time the needle was penetrating through the skin and after the injection, the following technic has been utilized: of body weight was 4.2, 3.0, or 2.1 mg of urethan.

Depending upon the solution of urethan utilized, mice were given injections each time of 0.7, 0.5, or 0.35 mg of this agent/gm body weight. Therefore, the total administered dose in mg/gm of body weight was around it, and the needle was removed.

The 2nd column under the heading “Effective No.” gives the number of mice that reached the weaning age (30 ± 3 days). The acute toxicity effects of urethan treatment on the survival rates were manifested only during the period prior to weaning. As expected, there was a direct relationship between the urethan dose and the mortality. Also, a higher mortality was observed in groups that received the 1st injection when less than 24 hr of age and subsequently at 3-day intervals. The greatest difference in the incidence of leukemias due to the age was observed in groups exposed to a 14% solution of urethan. Leukemias developed in 74% of newborns (Group 2) on the average of 19.7 weeks of age, while 7-day-old mice (Group 3) had an incidence of 38% at an average age of 20.5 weeks. When a 7% solution of urethan was utilized, 7% of newborn mice developed leukemias at an average age of 25.3 weeks.

The mortality rate in nontreated controls was only 5%.

Depending upon the age at the time of the initiation of the treatment and dose of urethan given, animals developed malignant lymphoma or leukemia in varied incidences. The thymus was the only organ which was involved in all instances. Its weight at the autopsy ranged from 160 to 1270 mg. Other organs were also involved but with lower frequency. The largest liver weighed 4860 and the largest kidney 770 mg. The spleen weight never exceeded 1000 mg. Enlargement of the superficial lymph nodes was a very frequent finding and was usually accompanied by enlarged mesenteric lymph node reaching a weight as high as 1600 mg. Not a rare finding was infiltration of testes by neoplastic cells. Several photomicrographs illustrate involvement of the lung, kidney, adrenal, liver, and testis (Figs. 1-4).

AGE-RENEWON RELATIONSHIP. It is obvious from Table 1 that at all 3 dose levels used, the incidence of leukemia was significantly higher in the groups in which the treatment began at newborn age (less than 24 hr post partum). The greatest difference in the incidence of leukemias due to the age was observed in groups exposed to a 14% solution of urethan. Leukemias developed in 74% of newborns (Group 2) on the average of 19.7 weeks of age, while 7-day-old mice (Group 3) had an incidence of 38% at an average age of 20.5 weeks. When a 7% solution of urethan was utilized, 7% of newborn mice developed leukemias at an average age of 25.3 weeks.

DOSE-RESPONSE RELATIONSHIP. Groups 2, 4, and 6 received 6 injections of 14, 10, or 7% solution of urethan, respectively. In all 3 instances, the 1st injection was given when the mice were less than 24 hr of age and subsequently at 3-day intervals. The total amount of urethan delivered/gm body weight for these 3 groups was 4.2, 3.0, and 2.1 mg. The incidence of leukemia was dose-dependent and varied significantly from group to group. Similar dose-response relationship was observed for 7-day-old groups (Groups 3, 5, and 7, Column 7).

In Chart 1 the percentages of mice that developed leukemias were transformed to degrees (angular transformation), and these were plotted against the total amounts of urethan given/gm body weight. The straight lines were fitted to these points by the least square method.

Test for linearity indicated existence of such a relationship between the dose of urethan and the incidence of leukemias for both age groups. The regression lines were significantly apart.

### Table 1

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CS/BL x C57 F1 mice</th>
<th>ADMINISTRATION OF URETHAN</th>
<th>MICE WITH LEUKEMIA</th>
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<td>1</td>
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<td>14</td>
<td>0</td>
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<td>14</td>
<td>83</td>
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</tr>
<tr>
<td>7</td>
<td>92</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

* Both sexes were equally distributed within the groups.

* Of the specified urethan solution, 0.005 ml was given/gm body weight. Each mouse received 6 i.p. injections at 3-day intervals.

* Animals alive at weaning.

* Age at recognition of leukemia—clinically and/or at postmortem examination.
Newborn Age and Urethan in Leukemogenesis

The outer zone of thymic cortex in newborn mice is mainly composed of large and immature lymphoid cells (1, 12). There is a correlation between the number of these cells in C3Hf/Bi mice and their susceptibility to lymphoma induction by Gross passage A virus (1). Kaplan was the first to suggest that impairment of differentiation of thymic cells contributes to the development of lymphoma (3, 11). However, the thymic injury following a single urethan treatment is short-lasting (9, 10) and not age-dependent (10). Its regeneration can be delayed by concurrent injury of the bone marrow. Haran-Ghera and Kaplan (10) demonstrated that the bone marrow of 30-day-old C57HL mice, but not of the older animals, is sensitive to urethan injury so that it loses in part its capacity to promote thymic regeneration. The authors then concluded that the absence of bone marrow injury might explain the diminished capacity of this agent to act as a complete leukemogen in older mice. Does the intensity of urethan injury to bone marrow gradually diminish within the first 30 days of life so that this might contribute to the lower leukemogenic response of infant mice to this agent? This is a possibility which appears worthy of consideration.

Apparently, Swiss newborn mice have particular sensitivity to urethan leukemogenesis by a single injection (8, 24) while a number of other strains are resistant (30). It is likely that positive results observed in the present experiments were partly due to repeated and periodic urethan treatments which sustained impairment of thymic recovery both by its direct action and indirectly through the bone marrow injury.

Recently, Miller et al. (20) demonstrated that mice thymectomized at 3 days of age were more responsive to the induction of skin tumors by 3,4-benzopyrene than the sham-thymec tomized animals. Similarly, viral carcinogenesis has been enhanced by neonatal thymectomy (19, 22). The thymectomy of the newborn animal prevents complete development of their immunologic competence (21). Does neonatal thymic injury by urethan delay the development of immunologic competence? If so, one should not exclude the possibility that immunologic incompetence of newborn animals might be one of the factors favoring leukemogenesis.

Therefore, the greater susceptibility of newborns to urethan leukemogenesis may be attributed in part to the slower catabolism of the urethan, the presence of the immature cells in the thymus and delayed thymic regeneration due to the concurrent bone marrow injury and possibly to immunologic incompetence. Further work and clarification is anticipated.

Acknowledgments

The authors wish to thank Dr. P. Shubik for his interest in the work and to Dr. L. Fiore-Donati for stimulating discussions. Thanks are also extended to Dr. M. Greenblatt for his suggestions regarding the photomicrographs.

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Fig. 1. Malignant lymphoid cells surround large and small radicals of the pulmonary vessels in the region of the bronchi. The pul-
monary parenchyma is otherwise unaffected. H & E, × 20.

Fig. 2. Neoplastic cells infiltrate the renal cortex separating and partially destroying parenchymal elements (lower portion). The
adrenal gland shows neoplastic lymphoid infiltration occurring in bandlike fashion within the subcapsular portion of the adrenal cor-
text and in more spotty fashion, within the cortical parenchyma (upper portion). H & E, × 70.

Fig. 3. A perivascular location of the infiltrate is seen surrounding radicals of the portal and hepatic veins. Malignant lymphoid
cells are also found intravascularly. Clusters of neoplastic cells are present within the parenchyma chiefly within the sinusoids. H &
E, × 70.

Fig. 4. The testicular tubules are surrounded and separated by the malignant infiltrate. Spermatogenesis is unaffected. H & E,
× 175.
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