# Leukemogenic Effect of Myleran on the Mouse Thymus. (26966)

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Of various alkylating agents with radiomimetic activity, Myleran (1,4-dimethanesulfonoxybutane) is particularly toxic to myelopietic cells(1). This property, which has led to the widespread use of Myleran in treatment of myeloid leukemia(2), suggested to us that the drug might be useful in conjunction with ionizing radiation for studying the pathogenesis of radiation-induced leukemia; *i.e.*, by means of Myleran, the marrow might be rendered aplastic before or after irradiation to determine the influence of myelopoietic activity on leukemia-induction. This premise formed the basis for the experiments herein reported.

*Methods.* Random-bred RF/Up male mice were exposed to 250 kvp whole-body X radiation at 10 weeks of age. The conditions of irradiation were identical to those reported earlier(3). Myleran was administered before or after irradiation (Table I), 20 mg/kg intravenously in a solution of dimethylacetamide, prepared according to the method of Weston *et al.*(4, and personal communication). The timing of Myleran administration in relation to irradiation was adjusted primarily so that the marrow was irradiated either at time of maximum aplasia (5 days after Myleran) or maximum regenerative hyperplasia (15 days after Myleran), as judged from preliminary histologic studies of mice treated identically. For comparison, irradiation was also carried out 5 days before Myleran administration. Control groups were sham-irradiated and injected with dimethylacetamide alone (the Myleran vehicle). After treatment, the animals were caged in groups of 9-10, with free access to Purina laboratory chow and drinking water, and observed until natural death. A necropsy was performed on each animal, with histologic examination as needed to confirm gross diagnosis.

*Results.* Mortality within the first 30 days after treatment was negligible in all groups. Exposure to X-rays shortened the ultimate life span, however, and increased the incidence of myeloid leukemia and thymic lymphosarcoma, the effects of combined treatment with X-rays and dimethylacetamide (Table I) closely approximating those obtained previously with X-rays alone(3). Myleran, whether administered alone or in conjunction with X-rays, reduced survival time and incidence of myeloid leukemia and increased the incidence of thymic lymphoma, but the magnitude of these effects varied with conditions of treatment (Table I); *i.e.*, it was most pronounced when Myleran was administered 15 days before irradiation (Fig. 1). The Myleran vehicle, dimethylacetamide, was without significant effects on the life span or incidence of these neoplasms. Incidence of leukemias other than the myeloid and thymic types tended to be lower among irradiated mice than among controls, in agreement with earlier observations(3), and was not significantly affected by Myleran. The mean age at death with all observed diseases was reduced by irradiation, in keeping with previous findings(3).

*Discussion.* The co-leukemogenic, and possibly leukemogenic, effects of Myleran on the thymus were not anticipated at the outset of the study. Instead, it was expected that Myleran would probably enhance induction of myeloid leukemia because of its toxicity to the marrow, an effect opposite to that obtained. This paradox is yet to be explained, but several factors deserve consideration.

First, it is conceivable that Myleran caused too much injury to myelopoietic cells to permit myeloid leukemogenesis, since excessive injury by X-rays alone (*i.e.*, doses above 400 r) inhibits the induction of the disease(3). The preponderantly higher incidence of myeloid leukemia in the Myleran-treated mice at 300 r than at 150 r, however, is inconsistent with the idea that the inhibition resulted from toxicity alone. Furthermore, the inhibitory

* Operated by Union Carbide Corp. for the U. S. Atomic Energy Commission.

TABLE I. Leukemia Incidence as Affected by Myleran and X-Rays.

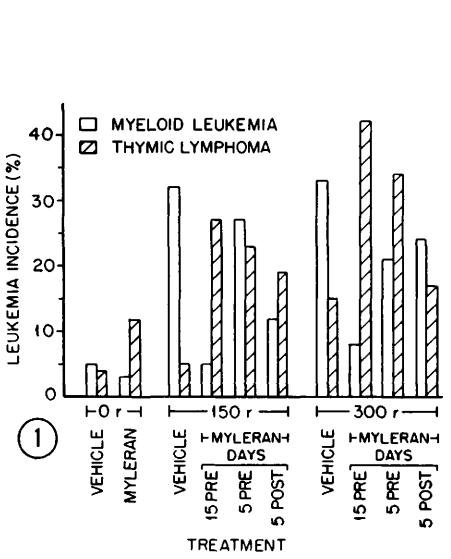
| Treatment | No. of mice | Mice dying with myeloid leukemia | | | Mice dying with thymic lymphoma | | | Mice dying with other leukemia | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean age at death (mos) | Incidence Observed (%) | Incidence Adjusted[a] (%) | Mean age at death (mos) | Incidence Observed (%) | Incidence Adjusted[b] (%) | Mean age at death (mos) | Incidence observed (%) | Mean age at death (mos) |
| None (control)[c] | 314 | 19.1 | 3 | 4 (± 1) | 16.3 | 5 | 5 (± 3) | 15.5 | 29 | 20.6 |
| Vehicle | 71 | 20.1 | 5 | 5 (± 4) | 16.0 | 4 | 4 (± 4) | 18.0 | 45 | 19.2 |
| Myleran | 59 | 17.2 | 2 | 2 (± 2) | 20.0 | 12 | 12 (± 5) | 10.9 | 39 | 19.0 |
| 150 r + vehicle | 74 | 16.0 | 32 | 33 (± 5) | 15.5 | 5 | 6 (± 4) | 10.5 | 23 | 16.4 |
| 150 r + Myleran 15 days before | 67 | 14.9 | 5 | 8 (± 5) | 13.3 | 27 | 28 (± 6) | 9.0 | 29 | 17.1 |
| " " " 5 " " | 71 | 13.6 | 27 | 34 (± 6) | 13.4 | 23 | 26 (± 6) | 10.1 | 19 | 15.2 |
| " " " 5 " after | 69 | 14.8 | 11 | 14 (± 6) | 15.4 | 19 | 20 (± 6) | 9.7 | 32 | 15.6 |
| 300 r + vehicle | 70 | 13.7 | 33 | 38 (± 5) | 12.8 | 15 | 17 (± 6) | 9.4 | 18 | 17.7 |
| " " Myleran 15 days before | 70 | 12.6 | 8 | 14 (± 7) | 12.3 | 42 | 44 (± 7) | 9.5 | 20 | 16.1 |
| " " " 5 " " | 71 | 13.6 | 21 | 31 (± 7) | 13.0 | 34 | 38 (± 4) | 9.4 | 14 | 14.7 |
| " " " 5 " after | 74 | 15.2 | 24 | 28 (± 6) | 13.7 | 17 | 19 (± 6) | 11.5 | 23 | 16.6 |

[a] Observed incidence adjusted(5) to correct for loss of animals attributed to mortality from thymic lymphoma (standard error in parentheses).
[b] " " " " " " " " " Myeloid leukemia " " " "
[c] Controls from earlier experiments(3).

effect of Myleran varied with time of administration, being maximal when the drug was given 15 days before irradiation. Another possible explanation for the inhibition was the high frequency of Myleran-induced thymic lymphosarcoma, since the incidence of this disease tended to be inversely related to the incidence of myeloid leukemia. Statistical adjustment of the myeloid leukemia incidence to correct for this possibility, however, failed to restore it to expected levels (Table I). An additional explanation of the effects observed when Myleran was given 15 days before irradiation is that Myleran may have increased the number of myeloblasts surviving irradiation by increasing the total number irradiated; *i.e.,* the marrow was rendered maximally hyperplastic at time of irradiation. This mechanism fails, however, to account for the effects of Myleran when given after irradiation.

Equally puzzling is the co-leukemogenic action of Myleran on the thymus, which also varied in relation to the time between Myleran administration and irradiation. Owing to the protective activity of intact marrow in inhibiting radiation-induced thymic tumorigenesis(see 6), and the co-leukemogenic action of urethane when given in conjunction with X-rays and marrow shielding(7), the effects of Myleran may be postulated to have resulted from marrow toxicity. It is noteworthy, however, that the action of Myleran was maximal when the drug was given 15 days before irradiation, whereas in the case of urethane, co-leukemogenic activity is minimal when the agent is given before irradiation(8). Although the 2 agents thus far have not been compared under identical experimental conditions, the data suggest that the leukemogenic effects of urethane and Myleran differ in mechanism. Thus, a more detailed analysis of the leukemogenic activity of the 2 agents in relation to the pharmacologic and toxicologic effects is called for.

We made an effort to ascertain whether Myleran alone caused thymic involution and whether it would impair regeneration of the thymus when given in conjunction with X-rays under the conditions of this experiment. This inquiry was prompted by observations



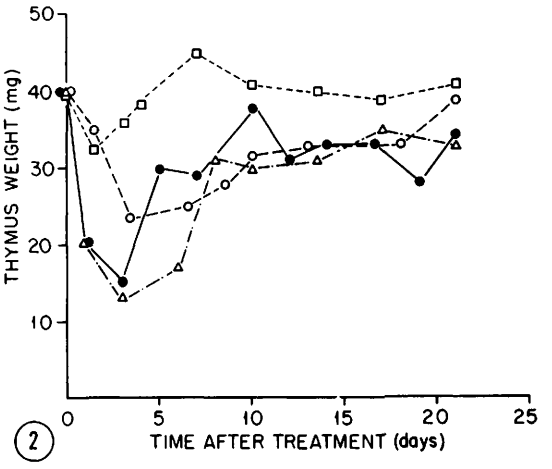




FIG. 1. Observed incidence of myeloid leukemia and thymic lymphoma as influenced by time of Myleran injection in relation to irradiation.

FIG. 2. Influence of Myleran on thymus weight and restoration of thymus weight after irradiation (each point represents data for 6 mice; see text for details of treatment).
□ vehicle
○ Myleran, 20 mg/kg
△ 300 r whole-body X-irradiation
● Myleran, 20 mg/kg, 15 days before 300 r whole-body X-irradiation

that thymic tumorigenesis appears to be inversely correlated with the rapidity of restoration of thymus weight after irradiation(9) and that urethane decreases the ability of the marrow to promote regeneration of the irradiated thymus(10). From the limited data available (Fig. 2), there is no evidence that Myleran adversely affected thymus regeneration at the dose level used in this study, although it did cause transient atrophy. Since, however, larger doses of Myleran have been observed to cause more profound and long-lived thymic atrophy(11), the limited thymic weight data presented herein should not be regarded as conclusive.

*Summary.* Myleran given before or after whole-body X-irradiation increased the incidence of thymic lymphomas and decreased the incidence of myeloid leukemias in male mice of the RF strain. The magnitude of these effects varied, depending on the time Myleran was administered in relation to irradiation. Effects were greatest when Myleran was given 15 days before exposure to X rays.

These results are paradoxical in view of the myelotoxic action of Myleran and attest further to the complexity of factors influencing susceptibility to radiogenic leukemia.

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1. Haddow, A., Timmis, G. M., *Lancet,* 1953, v264, 207.

2. Galton, D. A. G., Till, M., *ibid.,* 1955, v268, 425.

3. Upton, A. C., Wolff, F. F., Furth, J., Kimball, A. W., *Cancer Res.,* 1958, v18, 842.

4. Weston, J. K., Maxwell, R. E., Lee, M., Finzel, J., Frisken, R. A., *Fed. Proc.,* 1957, v16, 377.

5. Kimball, A. W., *Bull. Internat. Statist. Inst.,* 1958, v36, 193.

6. Kaplan, H. S., *Cancer Res.,* 1959, v19, 791.

7. Kawamoto, S., Ida, N., Kirschbaum, A., Taylor, G., *ibid.,* 1958, v18, 725.

8. Berenblum, I., Trainin, N., *Science,* 1960, v132, 40.

9. Kaplan, H. S., Brown, M. B., in *The Leukemias: Etiology, Pathophysiology, and Treatment,* Eds.,

J. W. Rebuck *et al.*, 1957, Academic Press, Inc., 163.

10. Berman, I., Kaplan, H. S., *Radiation Res.*, 1959, v11, 24.

11. Asano, M., Odell, T. T., Jr., Upton, A. C., unpublished data.

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## Behavior of Different Geographic Strains of *Schistosoma japonicum* as Determined by the Circumoval Test.* (26967)

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Species specificity of *Schistosoma haematobium, S. mansoni,* and *S. japonicum* has been demonstrated serologically by the circumoval test(1). As *S. japonicum* consists of at least 4 distinct geographic strains, Chinese, Formosan, Japanese, and Philippine(2), it is of interest to know whether these 4 strains are distinct in the circumoval test.

*Materials and methods.* Four geographic strains of *S. japonicum* were used: the Chinese (C), Formosan (F), Japanese (J), and Philippine (P). The cercariae of the C strain were obtained from *Oncomelania hupensis* originating in Kashing, Chekiang, China; the F strain, from *O. formosana* obtained from Changhua, Taiwan, China; the J strain, from *O. nosophora* obtained from Kofu, Japan; and the P strain, from *O. quadrasi* obtained from Leyte, in the Philippines.

Eight rabbits divided into 4 groups of 2 each were infected by the cutaneous route with the 4 strains of *S. japonicum.* Each rabbit was infected with 70 male and 70 female cercariae of a strain of the parasite and was bled on the 70th day after schistosome eggs were found in the stools. The sera obtained from these 8 rabbits were the antisera (AS) used in the circumoval test. Sera from 2 normal rabbits were used as controls. The antisera and normal sera (NS) were inactivated at 56°C for 30 minutes before use.

The eggs of *S. japonicum* were obtained from the liver of infected mice. Twenty-four mice divided into 4 groups of 6 each were infected with the 4 strains of *S. japonicum.* Each mouse was exposed to 100 cercariae of balanced sexes of one strain of *S. japonicum.* These mice were sacrificed on the fifth day after eggs were found in stools. The livers of the mice were ground and schistosome eggs were then concentrated by repeated natural sedimentation with normal saline. The last concentration was accomplished by centrifugation.

Single drops containing these centrifuged eggs were then mixed with single drops of either antiserum or normal serum on a slide. These serum-egg mixtures were then covered with 22 mm square coverglasses and rimmed with paraffin. The slides were kept at 37°C in an incubator, and the results were read after 24 hours' incubation. Only the eggs (E) which contained active mature miracidium were recorded as the number of eggs examined in this test. The antigen-antiserum precipitates which appeared on the egg surface were carefully differentiated from the other precipitates noted occurring with normal serum. Only the former were recorded as eggs with positive reactions. As the degrees of the positive reactions are prone to subjective errors, they were not classified in this study.

In the accompanying table, the following abbreviations are used: CE refers to eggs of Chinese strain; FE, the Formosan strain; JE, the Japanese strain; PE, the Philippine strain. CAS refers to antiserum of the Chinese strain; FAS, the Formosan strain; JAS, the Japanese strain; and PAS, the Philippine strain.

*Results.* Twenty sets of egg-serum tests, divided into 4 series, were made. The results

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