Influence of Colchicine on Leukemogenic Effect of X-Ray, Estrogen, Methylcholanthrene, and Urethan in Mice*

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SUMMARY

Colchicine had a significant inhibitory influence on the leukemogenic effect of estrogenic hormone in combination with either x-ray or urethan in mice. There was some suggestion that colchicine inhibited the induction of leukemia in mice by x-rays.

Colchicine was not leukemogenic in low-leukemia strains of mice when administered alone. Colchicine did not affect spontaneous (presumably viral) leukemogenesis in AKR mice.

Both urethan and colchicine are "radiomimetic" drugs (2, 3), with distinct depressive action on the hemopoietic tissues (11, 19). Colchicine derivatives and urethan have been used in the treatment of chronic myeloid leukemia (12, 15). Almost complete regression of transplanted lymphoid tumors in mice has been induced by colchicine (9).

Recently, pretreatment with colchicine has been reported to accelerate hemopoietic recovery in irradiated mice (17). The results of a number of oncological studies of urethan itself or a combination of urethan with other carcinogens in mice have been published (1, 14, 16, 18). In 1958, we reported that urethan augmented leukemogenesis in mice by x-ray, estrogenic hormone, and methylcholanthrene, although urethan was not leukemogenic in mice when administered independently (6). These experiments were undertaken to determine whether or not colchicine would have an effect on mouse leukemogenesis similar to that of urethan.

MATERIALS AND METHODS

Colchicine was administered independently or in various combinations with x-rays, estrogen, methylcholanthrene, and urethan.

Inbred C57BL, DBA/2, and BALB/c mice and (C57BL × C3H) F₁ hybrid mice were used. All these stocks are considered to be "low-leukemia" strains from the standpoint of spontaneous occurrence of leukemia. High-leukemia strain AKR mice also were treated with colchicine alone to study the influence of this chemical on spontaneous leukemia of presumably viral etiology.

Administration of colchicine alone to various strains of mice.—Colchicine was given independently to mice in treatment schedules similar to that of urethan in previous experiments (6). Colchicine was injected subcutaneously in eleven doses of 1 μg/gm of mouse weight in 0.02 per cent aqueous solution at 4-day intervals to low-leukemia strains of mice, beginning at 6 weeks of age. The experimental animals were observed for over 2 years after treatment.

Colchicine, administered alone, was not leukemogenic for either males or females of the low-leukemia strains C57BL, DBA/2, BALB/c, and (C57BL × C3H) F₁ (Table 1).

Colchicine was also administered in the same dose to AKR mice, beginning at weaning age. Colchicine did not significantly alter the incidence or age of onset of spontaneous leukemia of presumably viral etiology, in either male or female mice of the AKR strain (Chart 1).

Colchicine combined with x-ray.—The effect of colchicine on leukemogenesis by x-ray was tested with BALB/c and DBA/2 male mice and (C57BL × C3H) F₁ hybrid mice of both sexes (Table 2). In the latter instance, equal numbers of males and females were employed, one sex not outnum-
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The dosage of colchicine was 1 µg/gm of mouse weight (0.02 per cent aqueous solution). The initial dose was given within 1 hour after the first dose of x-radiation. The animals were divided into the following two treatment groups:

Group 1: 90 r of whole-body x-ray, 4 times at 4-day intervals, no colchicine.¹

Group 2: Same treatment with x-ray, plus administration of colchicine, 11 times.

These strains of mice were known to be susceptible to the induction of leukemia by x-ray (4, 6, 7).

In the same manner, C57BL mice of both sexes were also studied to test the influence of colchicine on leukemogenesis by x-ray and urethan in mice (Table 2). Equal numbers of males and females were used, one sex not outnumbering the other sex by more than two mice in any group. The following treatment groups were used:

Group 1: 40 r of whole-body x-ray every 4th day, 11 times, no colchicine and no urethan.

Group 2: Same treatment with x-ray, plus colchicine, given after each x-radiation, no urethan.

Group 3: Same treatment with x-ray under urethan anesthesia each time (1 mg/gm of mouse weight, administered intraperitoneally), no colchicine.

Group 4: Same treatment as Group 3, plus administration of colchicine after x-radiation.

¹ Physical factors: 140 kvp, 5 ma., 2.0 mm. Al added filter, 30 cm. target to mouse distance. Output, 58.8 r/min.

### TABLE 1

INCIDENCE OF LEUKEMIA IN COLCHICINE-TREATED AND UNTREATED MICE OF SEVERAL STRAINS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Strain</th>
<th>Sex</th>
<th>No. mice</th>
<th>No. developing leukemia</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>C57BL</td>
<td>F</td>
<td>148</td>
<td>13</td>
<td>8.8</td>
</tr>
<tr>
<td>None</td>
<td>(C57BLXCSH)F1</td>
<td>F</td>
<td>46</td>
<td>4</td>
<td>8.7</td>
</tr>
<tr>
<td>Colchicine*</td>
<td>C57BL</td>
<td>F</td>
<td>12</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td>Colchicine</td>
<td>(C57BLXCSH)F1</td>
<td>M</td>
<td>13</td>
<td>2</td>
<td>13.3</td>
</tr>
<tr>
<td>Colchicine</td>
<td>DBA/2</td>
<td>M</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Colchicine</td>
<td>BALB/c</td>
<td>M</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* 1 µg/gm of body weight every 4th day, 11 times.
Irradiation treatment alone resulted in an incidence of leukemia ranging from 21.9 to 36 per cent for the four strains of mice (Table 2). Combined colchicine and irradiation treatment resulted in a uniformly decreased incidence of leukemia that did not reach statistically significant levels except in the DBA/2 strain where, however, six mice are still alive and apparently nonleukemic at ages only slightly beyond the average age at death from leukemia for the irradiated controls.

Combined treatment of C57BL mice with irradiation and urethan resulted in 39.2 per cent leukemia. The addition of colchicine treatment delayed the onset of leukemic deaths and reduced the incidence to 18.5 per cent, approaching significance at the .05 level. Seven mice of this group are still alive and nonleukemic at ages well beyond the mean age of leukemic deaths.

Colchicine combined with estrogen and urethan.—Estrogenic hormone was mildly leukemogenic, whereas combined estrogen and urethan significantly increased the incidence of leukemia in C57BL mice (6). In the present study the effect of added colchicine on each of these treatments in castrated male C57BL mice was determined. The treatments were as follows (Table 3):

<table>
<thead>
<tr>
<th>Group 1:</th>
<th>25 µg. of estrogenic hormone(a) in peanut oil given once a week for 40 weeks, intramuscularly, beginning at 6 weeks of age; no colchicine and no urethan.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2:</td>
<td>Same treatment as Group 1, plus colchicine every 4th day, 11 times, beginning after the first administration of estrogenic hormone; no urethan.</td>
</tr>
<tr>
<td>Group 3:</td>
<td>Same treatment as Group 1, plus urethan (1 mg/gm of body weight) every 4th day, 11 times; no colchicine.</td>
</tr>
<tr>
<td>Group 4:</td>
<td>Same treatment as Group 3, plus colchicine.</td>
</tr>
</tbody>
</table>

In both instances colchicine appreciably delayed the mean age of leukemic deaths. When combined with estrogen and urethan treatment the incidence of leukemia was significantly reduced. Seven mice of this group are still alive and nonleukemic at ages well beyond the mean age of leukemic deaths.

TABLE 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>No. of Mice</th>
<th>Leukemia</th>
<th>2*</th>
<th>P Value</th>
<th>Mean Age and Nonleukemic No.</th>
<th>Mean Age and Nonleukemic No.</th>
<th>Alive</th>
<th>Nonleukemic</th>
<th>Alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c (intact male)</td>
<td>(90rX4)†</td>
<td>25</td>
<td>9</td>
<td>36.0</td>
<td>.02</td>
<td>483 (188-569)</td>
<td>16</td>
<td>545</td>
<td>187-776</td>
<td>507</td>
</tr>
<tr>
<td>+colchicine†</td>
<td>30</td>
<td>5</td>
<td>16.7</td>
<td>.2</td>
<td></td>
<td>353 (300-630)</td>
<td>25</td>
<td>371</td>
<td>368-572</td>
<td>0</td>
</tr>
<tr>
<td>DBA/2 (intact male)</td>
<td>(90rX4)</td>
<td>32</td>
<td>7</td>
<td>21.9</td>
<td>.05-02</td>
<td>365 (314-385)</td>
<td>25</td>
<td>537</td>
<td>126-705</td>
<td>415</td>
</tr>
<tr>
<td>+colchicine</td>
<td>24</td>
<td>4</td>
<td>16.0</td>
<td>.02</td>
<td></td>
<td>363 (144-587)</td>
<td>18</td>
<td>413</td>
<td>126-705</td>
<td>371</td>
</tr>
<tr>
<td>C57BL x C3H (F1 hybrid male and female)§</td>
<td>(90rX4)</td>
<td>28</td>
<td>9</td>
<td>32.1</td>
<td>.2-1</td>
<td>655 (371-727)</td>
<td>19</td>
<td>677</td>
<td>446-871</td>
<td>0</td>
</tr>
<tr>
<td>+colchicine</td>
<td>24</td>
<td>3</td>
<td>12.5</td>
<td>.1</td>
<td></td>
<td>621 (517-707)</td>
<td>21</td>
<td>646</td>
<td>359-884</td>
<td>0</td>
</tr>
<tr>
<td>C57BL (intact male and female)§</td>
<td>(40rX11)</td>
<td>34</td>
<td>8</td>
<td>23.5</td>
<td>.1</td>
<td>614 (259-765)</td>
<td>26</td>
<td>696</td>
<td>180-883</td>
<td>0</td>
</tr>
<tr>
<td>+colchicine</td>
<td>27</td>
<td>3</td>
<td>11.1</td>
<td>.05</td>
<td></td>
<td>357 (244-518)</td>
<td>24</td>
<td>727</td>
<td>392-592</td>
<td>0</td>
</tr>
<tr>
<td>+urethan#</td>
<td>51</td>
<td>20</td>
<td>39.2</td>
<td>.1</td>
<td></td>
<td>280 (140-487)</td>
<td>31</td>
<td>497</td>
<td>113-900</td>
<td>0</td>
</tr>
<tr>
<td>+urethan + colchicine</td>
<td>27</td>
<td>5</td>
<td>18.5</td>
<td>.05</td>
<td></td>
<td>344 (168-512)</td>
<td>15</td>
<td>456</td>
<td>282-611</td>
<td>7</td>
</tr>
</tbody>
</table>

* In calculation of χ2, the correction factor was used whenever there were less than five mice in any of the four expected classes.
† Whole-body x-radiation. Physical factors: 140 kVp, 5 ma., 2.0 mm. Al added filter, 30 cm. target to mouse distance. Output, 88.8 r/min.
‡ Colchicine, 1 µg/gm body weight, every 4th day, 11 times, started immediately after the first x-radiation.
§ Equal numbers of males and females were used, one sex not outnumbering the other sex by more than two mice in any group.
# Urethan 1 mg/gm body weight given intraperitoneally at 4-day intervals, for 11 times, before x-radiation.
Colchicine combined with both x-ray and estrogenic hormone.—In this experiment, to test the protective effect of colchicine against leukemogenesis induced by the synergistic action of x-ray with estrogenic hormone (8), the following groups of castrated (C57BL × C3H) F1 hybrid male mice were utilized (Table 3):

Group 1: 90 r of whole-body x-ray every 4th day, 4 times, plus 25 μg. of estrogenic hormone weekly for 40 weeks, beginning at 6 weeks of age; no colchicine.

Group 2: Same treatment as Group 1, plus colchicine every 4th day, 11 times, starting immediately after the first x-radiation.

In mice receiving both leukemogenic agents, x-ray, and estrogenic hormone, the incidence of leukemia was 100 per cent (26/26). The leukemia incidence was significantly reduced to 47.8 per cent in mice receiving colchicine in addition to x-ray and estrogenic hormone as shown in Table 3 ($x^2 = 15.2; P < .001$).

Colchicine combined with methylcholanthrene.—Methylcholanthrene is well known as a leukemogen when painted on the skin of some strains of mice, especially the DBA/2 strain (13). Two groups of DBA/2 male mice were treated as follows (Table 3):

Group 1: Methylcholanthrene (0.25 per cent solution in benzene) painted on different skin sites, 3 times a week for 9 times, beginning at 6 weeks of age; no colchicine.

Group 2: Same treatment as group 1, plus administration of colchicine every 4th day, 11 times, starting after the first painting with methylcholanthrene.

When colchicine was administered with methylcholanthrene, the onset of leukemia was delayed, and the final incidence of leukemia slightly reduced.

DISCUSSION

Colchicine and urethan are radiomimetic agents. Urethan, while not independently leukemogenic, augments leukemogenesis in mice by x-ray, estrogen, and methylcholanthrene (6). In the present

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>TREATMENT</th>
<th>NO. MICE</th>
<th>LEUKEMIA</th>
<th>$x^2$</th>
<th>P VALUE</th>
<th>MEAN AGE AND (RANGE) AT DEATH FROM LEUKEMIA IN DAYS</th>
<th>MEAN AGE AND (RANGE) AT DEATH FROM NONLEUKEMIC IN DAYS</th>
<th>ALIVE NONLEUKEMICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL* (castrated male)</td>
<td>Estrogen†</td>
<td>30</td>
<td>6</td>
<td>20.0</td>
<td>.4</td>
<td>316 (161-462)</td>
<td>24 (118-713)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Estrogen+colchicine†</td>
<td>21</td>
<td>2</td>
<td>9.5</td>
<td>.5</td>
<td>451 (396-577)</td>
<td>14 (307-475)</td>
<td>5 (697-694)</td>
</tr>
<tr>
<td></td>
<td>Estrogen+urethan</td>
<td>22</td>
<td>5</td>
<td>22.7</td>
<td>6.07</td>
<td>435 (322-554)</td>
<td>10 (110-734)</td>
<td>7 (616-716)</td>
</tr>
<tr>
<td></td>
<td>Estrogen+urethan+colchicine</td>
<td>22</td>
<td>5</td>
<td>22.7</td>
<td>.02-01</td>
<td>435 (322-554)</td>
<td>10 (110-734)</td>
<td>7 (616-716)</td>
</tr>
<tr>
<td>C57BL×C3H† F1 hybrid (castrated male)</td>
<td>W. B. x-rad. (90 r×4)̸ + estrogen</td>
<td>26</td>
<td>26</td>
<td>100</td>
<td>15.2</td>
<td>237 (151-610)</td>
<td>12 (118-713)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>W. B. x-rad. (90 r×4)̸ + estrogen+colchicine</td>
<td>23</td>
<td>11</td>
<td>47.8</td>
<td>&lt; .001</td>
<td>298 (178-605)</td>
<td>12 (139-635)</td>
<td>0</td>
</tr>
<tr>
<td>DBA/2 (intact male)</td>
<td>9×MCA</td>
<td></td>
<td></td>
<td>31</td>
<td>10</td>
<td>32.2</td>
<td>6.67</td>
<td>304 (165-462)</td>
</tr>
<tr>
<td></td>
<td>9×MCA+colchicine</td>
<td>26</td>
<td>5</td>
<td>19.2</td>
<td>.5-3</td>
<td>401 (253-551)</td>
<td>21 (378-635)</td>
<td>0</td>
</tr>
</tbody>
</table>

Treatment was begun at 6 weeks of age.
* These animals were castrated at 4 weeks of age.
† 25 μg. estradiol dipropionate, injected intramuscularly, once a week, for 40 weeks starting at 6 weeks of age.
‡ Colchicine was injected subcutaneously in eleven doses every 4 days, beginning after the first treatment with estrogen.
§ Urethan, 1 mg/g body weight, injected intraperitoneally at 4-day intervals, for 11 times.
# Physical factors: 140 kvP, 5 ma., 2.0 mm. Al added filter, 30 cm. target to mouse distance. Output, 58.8 r/min.
|| Methylcholanthrene, 0.25 per cent solution in benzene, painted on different skin sites, 3 times a week for 3 weeks.
experiments colchicine was found not to be independently leukemogenic in four low-leukemia strains of mice, nor did it significantly alter the occurrence of spontaneous leukemia of presumably viral etiology in the AKR strain. In the present experiments, however, leukemogenesis by x-ray, estrogen, methylcholanthrene, or urethan, alone or in combination, was invariably reduced by colchicine administration. When the control incidence of induced leukemia was below 40 per cent, the extent of reduction by colchicine was slight and usually did not reach statistically significant levels for individual group comparisons by the $x^2$ method. When the control incidence of induced leukemia was 59 per cent (estrogen and urethan) and 100 per cent (estrogen and x-ray), the extent of reduction by colchicine reached highly significant levels. This decreased incidence of leukemia was not explicable on the basis of reduced longevity of the colchicine-treated animals. It appeared rather to be associated with a delay in the age of onset of leukemia.

The mechanism of this action of colchicine on leukemogenesis is open to speculation. However, Smith et al. (17) have reported that colchicine injected before a single dose of irradiation resulted in accelerated hemopoietic recovery comparable to that seen with postirradiation injection of isologous bone marrow (10). The latter, in turn, is known to suppress the development of irradiation-induced leukemia (5). In the present experiments colchicine was administered after each of a series of exposures to irradiation or injections of leukemogenic substances. By the same token, however, colchicine was administered before all but the first of the series of exposures or injections.

REFERENCES
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