A Sex Difference in the Mortality Pattern of L1210 Leukemia in DBA/2 Mice

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Abstract. Female DBA/2 mice inoculated with $1 \times 10^6$ L 1210 leukemia cells died more rapidly than simultaneously inoculated male mice with a mean survival of $6.1 \pm 0.2$ days vs. $7.2 \pm 0.4$ days. For males, this represents an average of 18% longer survival than females. Trials involving gonadectomy or treatment with exogenous sex hormones suggest that the sex difference in mortality was due to exacerbation of L 1210 leukemia by estrogen.

This report describes the effect of sex, gonadectomy and sex hormone treatment on mortality of DBA/2 mice carrying L 1210 transplantable murine leukemia. L 1210 is widely used as a screening entity in leukemia (Schabel et al 1966; Hofer and Hofer 1971). Recognition of a sex difference in the mortality pattern may be of value in improving the reliability of the test. A sex difference in susceptibility and resistance has been seen in a number of other diseases, including cancer (Gobe and Konopka 1973; Yohn 1973; Yohn et al 1967; Metcalf 1971).

METHODS AND MATERIALS

L 1210 Leukemia. L 1210 cells were maintained in DBA/2 mice using a 7 day transfer cycle. Mean weight of mice at time of use was $23.9 \pm 1.2$ g. One ml of ascites fluid was drawn from a donor mouse, diluted, and the white cells counted. The cell count was adjusted, if necessary, with sterile Locke's solution so that 0.1 ml contained approximately $1 \times 10^6$ cells. For experimental procedures, 0.1 ml of the adjusted solution was injected intraperitoneally. Sham inoculated animals (controls) were injected intraperitoneally with 0.1 ml of sterile Locke's solution. There were no deaths in any sham group. The mean survival time of the several groups of DBA/2 mice inoculated with $1 \times 10^6$ cells in this study varied between 6.1 and 7.2 days with an overall mean of $6.6 \pm 0.3$ days, in agreement with the known dynamics of L 1210 leukemia (Skipper et al 1964).

RESULTS

The cumulative percent mortality plot shows that female DBA/2 mice died significantly more rapidly than the males following inoculation with L 1210 cells (fig. 1). While mortality appeared similar on day 5, some 70% of the females,
as compared to 35% of the males, were
dead by day 6. The female mean sur-
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vival was 6.1 ±0.2 (SE) days while the
male mean survival was 7.2 ±0.4 days
(table 1). This difference was signifi-
cant at the p<0.01 level using a t-test. Us-
ing a probit plot of the pooled data, the
male LT₅₀ was estimated as day 6.7 post-
inooculation and the female LT₅₀ as day
5.5. This difference was also signifi-
cantly different (p<0.01) using a Chi Square
Test. While males lived only 1.1 to 1.2
days longer than females, on the average,
this amounts to an 18% increase in sur-
vival time.

After gonadectomy, the sex difference
in mortality was absent (table 1). The
mean survival time for both gonadecto-
mized males and females was 7.0 days
and the LT₅₀ was 6.5 days, similar to
normal males. After treatment with
exogenous hormones of the opposite sex,
the sex difference in mortality was again
absent (table 1). In this case, however,
the mean survival of 6.2 days and the
LT₅₀ of 5.5 days were similar to those of
normal females. Peanut oil alone did
not alter the mortality pattern.

**DISCUSSION**

These results suggest that the more
rapid mortality in female DBA/2 mice
inoculated with L 1210 leukemia cells
was due to an estrogenic effect. With
hormones lacking due to gonadectomy,
both males and females responded as
normal males. Thus, gonadectomized
females survived longer, but gonadec-
tomy did not affect mortality in the
males. In the procedure where mice
were treated with hormones of the op-
posite sex, both sexes responded as nor-
mal females. Thus, estrogen treated
males died sooner than normal males, but
androgen did not affect the mortality pat-
tern in the females. Estrogen thus ap-
peared to exacerbate (to some extent) the

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<th>Treatment</th>
<th>No. of trials</th>
<th>Total number of mice</th>
<th>Mean Survival (days±SE)</th>
<th>LT₅₀</th>
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<tr>
<td><strong>MALES</strong></td>
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<tr>
<td>L 1210 only</td>
<td>4</td>
<td>36</td>
<td>7.2±0.4</td>
<td>6.7*</td>
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<tr>
<td>Gonadectomy+L 1210</td>
<td>2</td>
<td>8</td>
<td>7.0±0.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Estrogen+L 1210**</td>
<td>2</td>
<td>10</td>
<td>6.2±0.2</td>
<td>5.5</td>
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<tr>
<td><strong>FEMALES</strong></td>
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<tr>
<td>L 1210 only</td>
<td>4</td>
<td>43</td>
<td>6.1±0.2</td>
<td>5.5</td>
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<tr>
<td>Gonadectomy+L 1210</td>
<td>2</td>
<td>8</td>
<td>7.0±0.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Androgen+L 1210†</td>
<td>2</td>
<td>10</td>
<td>6.2±0.3</td>
<td>5.5</td>
</tr>
</tbody>
</table>

*Significantly different from similarly treated females (p<0.01).
**Intraperitoneal injection of 125 µg Delestrogen (estradiol valerate), in peanut oil, S.C.
†Intraperitoneal injection of 5 mg Delatestryl (testosterone enanthate), in peanut oil, S.C.
L1210 leukemia disease process while androgens had little effect.

Estrogen has been implicated in other experimental cancers. Females are more susceptible to avian adenovirus, due to estrogenic enhancement of adenovirus oncogenesis (Jones et al 1970) and female hamsters develop tumors more readily, have a shorter survival time and show regression less often than male hamsters (Yohn 1973; Yohn et al 1967; Hatch et al 1970). In cell cultures, female hamster cells were transformed with adenovirus with a higher frequency than male cells, a result which was enhanced by the addition of estrogen (Fong and Ledinko 1970; Milo et al 1972). Enhancement of disease process in the female however, is far from universal. In infectious diseases caused by pneumococcus, the female was shown to be more resistant than the male (Weiss et al 1973). The sex difference in susceptibility to tumors induced with benzo(a)pyrene was shown to depend on the tissue involved (VesselNovitch et al 1975) and in leukemias involving AKR, RF and C58 mice, a sex difference exists but which sex is more susceptible depends upon the specific type of leukemia (Metcalf 1971).

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LITERATURE CITED


