Comparison of Simultaneously Incurred Damage to Bone Marrow and Tumor Tissue of Animals Treated with Anticancer Agents*

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SUMMARY

The suppression of tumor tissue (as measured by tumor weight) and the destruction of bone marrow (as measured by the number of nucleated cells per mouse femur) were determined at several dose levels of each of the compounds investigated.

The least reduction in the quantity of marrow cells occurred in the animals treated with 3',5'-dichloroaminopterin and mitomycin C. In contrast, marked suppression of tumor tissue was found in the same animals. Marked marrow and tumor suppression occurred in animals treated with thioTEPA. Mitomycin C was between dichloroaminopterin and thioTEPA in the separation of marrow and tumor damage. 6-Mercaptopurine caused the same effect on S-180 tumor tissue as on the bone marrow.

The daily changes in tumor weight and marrow count of animals treated with 5 mg/kg of thioTEPA/day for 6 days were described. Of these two tissues, tumor or marrow, the latter bore the brunt of the deleterious effects. The count was markedly lower on the day of harvest than that found on day zero. In contrast, the weight of the tumor tissue from treated animals was approximately the same as that initially found.

The volume of the femur cavity of the 18-gm. male C3H mouse was found to be 5.8 cu. mm., and the number of nucleated cells per femur was 9.5 (8.2-10.8) × 10⁶.

The efficient mechanics of screening for the detection of antineoplastic activity has revealed numerous compounds which can be labeled with the qualitative or descriptive term, "active." However, this designation does not convey quantitatively their comparative virtues, such as the effects they might have on the tumor-bearing host. Most of the screening procedures currently used, including that in this laboratory (1, 8), sacrifice quantitative answers for the necessary expediency of handling the ever-growing backlog of newly synthesized and untested compounds.

This paper is an attempt to refine the commonly used definition of host damage from the nonspecific parameters of total-body weight loss or death to the more specific parameter of damage to a host tissue; in this instance the bone marrow, and is presented as dose-response studies of this organ.

A simple and rapid way of collecting and quantitating the marrow was of primary importance. A number of methods found in the literature were considered. The method finally decided upon as the most efficient and accurate was that developed by Gerarde in which quantitative removal and counting of the nucleated cells of the femur marrow was used in a study of the toxic effects of benzene derivatives (4).

MATERIALS AND METHODS

GENERAL

All animals used in this study were C3H male mice weighing 18-20 gm. The technics of transplanting and harvesting the tumors (6C3HED lymphosarcoma, S-180, and the 72) mammary adenocarcinoma) were the same as those commonly used. The details of marrow removal and counting may be found in Gerarde’s study (4).

DOSE-RESPONSE STUDIES

A general description of the tumor and marrow tissue dose-response studies, which are the main part of this study, is given as a general schema.
Specific doses are stated in the text under "Results" and are entered on the abscissa of the charts. The highest nonfatal dose of the antineoplastic compound was used as the top dose. Additional lower doses were calculated by using a dose drop-page factor of 1.5 or 2.25. Groups of six animals per dose were used in testing compounds against the 72j mammary adenocarcinoma and S-180. These were selected by palpation for uniform tumor size after a growth period of 17 days or 6 days, respectively, and prior to placing the animals on test. Eight animals per dose were used in testing compounds against the 6C3HED lymphosarcoma (growth period of 3 days). Two or three replicates were performed on each compound. Animals were treated by intraperitoneal injection once a day for 6 days, and on the 7th day they were killed and the tumor weighed. Bone marrow nucleated cell counts were done on five animals for each group at each dose.

**RESULTS**

**VOLUME OF THE MARROW CAVITY**

The means of the various measurements are shown in Chart 1.

**NUMBER OF NUCLEATED CELLS PER FEMUR**

The mean number of nucleated cells was found to be 9.5 (8.2–10.8) X 10^6 cells per femur. Chart 2 shows a plot against time taken from control charts of mean counts (five animals per group) accumulated from the beginning of these studies. The time span from which these were taken is actually about 9 months; that depicted concerns the first and last 3 weeks. The counts obtained at the commencement of this work were below the lower confidence limits of the control chart. As more experience was gained by repeating the technique, all the mean counts fell between 8.2 and 10.8 X 10^6. It is felt that 9.5 X 10^6 is a reasonable estimate of the number of nucleated cells per femur of the 18–20-gm. male C3H mouse and that the somewhat lower earlier counts were due to techniques of harvesting and counting.
ThioTEPA: N,N',N"-Triethylene-thiophosphoramide

Dose response of marrow counts and tumor weights in animals treated with various doses of thioTEPA.

Mice bearing 73J mammary adenocarcinoma were given injections of the following doses of thioTEPA: 6, 4, 2.65, 1.78, and 1.18 mg/kg. This experiment was replicated 3 times (six animals per dose). Marrow counts and tumor weights were done on buffer-treated tumor-bearing animals for a total of eighteen animals.

The mean tumor weights and marrow counts and their 95 per cent confidence limits for the eighteen or fifteen animals, respectively. Control marrow and tumor mean values were 7(6.4--7.5) X 10^6 nucleated cells and 990 (560--1,800) mg, respectively.

Histological studies were not done on the tumor tissue, so it cannot be said that cellular damage to the neoplasm did not occur. If weight only is considered, neutralization of further growth, with accompanying sacrifice of the massive bone marrow damage, was the extent of the antineoplastic effect.

Mitomycin C

The dose responses against the 73J mammary adenocarcinoma and bone marrow with mitomycin C (11) were replicated 3 times at the following doses: .444, 1.0, and 2.25 mg/kg. Chart 6 shows a plot of the corrected means and their 95 per cent confidence limits, of the tumor weights and mar-
row counts. It can be seen that the slope of the marrow regression on dose was less than that occurring with thioTEPA, and greater than that with dichloroaminopterin (see Charts 4 and 7). The actual values of the slopes for the two tissues (tumor or marrow) were $-0.80 \pm 0.2$ and $-0.31 \pm 0.23$, respectively. The suppression of tumor growth was quite marked at the highest dose of 2.25 mg/kg. The marrow count at this dose was also suppressed but was considerably higher than that found with the highest dose of thioTEPA.

6-MERCAPTOPURINE

Two replicates of dose responses at 29.5, 66.7, and 150 mg/kg were run with this compound against mice bearing the Sarcoma 180 tumor. Ta-
3′,3″-Dichloroaminopterin

This compound was used at doses of 1.48, 3.34, and 7.5 mg/kg. The experiment was replicated 3 times against the 6C3HED tumor. Chart 7 is a plot of the regression lines of the marrow count and tumor weight against dose. The regression for the marrow count was moved by multiplying each ordinate value by .17 in order to move its origin to that of the tumor plot. There was moderate concomitant depression of the bone marrow, with marked suppression of the 6C3HED tumor growth with this antifolic acid congener.

DISCUSSION

The per cent of body weight accounted for by bone marrow has been determined in the rabbit (10), rat (2), dog (3), guinea pig (5,6), cat (7), and human (9). To date there is no direct information on the entire amount of marrow found in the mouse. The marrow should be thought of as an injured organ in experimental cancer chemotherapy, rather than as a single area, such as the femur. To do this several assumptions will have to be made:

1. The marrow weight of the C3H male mouse is 2 per cent of the total-body weight or the same as that reported for the rat and guinea pig.
2. The number of nucleated cell/cu mm of bone marrow in any body region is $5 \times 10^8/10^6$ cu mm = $1.8 \times 10^8$ cells.
3. The destructive changes in the marrow occur to an equivalent degree in all cavities.
4. The specific gravity of mouse marrow is 1.0.

The mice used in this study averaged 18 gm. and probably contained 360 mg. of bone marrow.

![Chart 7](chart-7.png)

**Chart 7.**—Plot of the 6C3HED tumor and marrow dose responses of mice treated with dichloroaminopterin. Doses ranged from 1.48 to 7.5 mg/kg/day for 6 days. Points represent the mean of fifteen or 24 animals for the marrow and tumor, respectively. Control mean marrow and tumor mean values were 10 (9.4-11) $\times 10^6$ cells and 1,900 (1,000-1,400) mg., respectively.

**TABLE 1**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Bone marrow cells (Millions)</th>
<th>Tumor weight (mg.)</th>
</tr>
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<tbody>
<tr>
<td>Buffer</td>
<td>10 (8.9-11.2)</td>
<td>1,770 (1,553-2,018)</td>
</tr>
<tr>
<td>29.5</td>
<td>6.31 (3.92-10.16)</td>
<td>883 (724-1,077)</td>
</tr>
<tr>
<td>66.7</td>
<td>5.47 (3.57-4.68)</td>
<td>543 (478-634)</td>
</tr>
<tr>
<td>150</td>
<td>1.97 (1.32-3.17)</td>
<td>355 (275-400)</td>
</tr>
</tbody>
</table>

**Bone Marrow Counts and Tumor Weights of Animals Treated with 6-Mercaptopurine**

Mean values and 95% confidence limits of marrow counts and tumor weights taken from animals treated with 6-mercaptopurine. Each point represents ten or twelve animals for the marrow and tumor, respectively.

The slope of the plots of the marrow counts obtained with thiotoTEPA and 6-mercaptopurine showed that the relative damage to the marrow was the same as that caused to the tumor growing in the same animals. The slopes shown by mitomycin indicated relatively greater tumor damage.
than marrow damage. This difference was most pronounced with dichloroaminopterin.

Work is being done in this laboratory to determine whether simultaneous marrow and tumor responses could be used as an aid in the evaluation of large numbers of active antineoplastic agents.

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REFERENCES

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