Infusion of Marrow in the Mouse and Dog after Thio-TEPA

By H. L. Lochte, Jr., S. Kasakura, M. Karetzky, Joseph W. Ferrebbee and E. Donnell Thomas

THE POSSIBILITY that the therapeutic range of chemotherapeutic agents may be extended by the use of infusions of isogeneic marrow to restore hematopoiesis has aroused considerable interest. Clinical reports have illustrated the difficulties of evaluating this form of treatment. In animals, Weston et al. have described restoration of marrow function in rats given Myleran. A slightly beneficial effect of isogeneic marrow has been observed in mice receiving HN₂, TEM, and thioguanine.

This report describes the use of fresh isogeneic marrow in the mouse and stored autologous marrow in the dog in attempts to modify the mortality that follows the administration of thio-TEPA.

MATERIALS AND METHODS

Ten- to 12-yeck-old male mice of the BDF₁ (C57Bl x DBA/²) strain weighing between 24 and 26 Gm. were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me., and housed 10 to a cage with food and water available ad lib. N, N', N"-Triethylenethiophosphoramide (Thio-TEPA) was obtained from Lederle Laboratories* and made into solutions of the desired concentration with a balanced salt solution. The volume of injection for the mice was 0.25 ml. given via the tail vein or intraperitoneally. Bone marrow was obtained from mice of the same hybrid type (BDF₁) and sex. The marrow from the femora and tibiae was flushed with TC-199 through a 20- and then a 25-gauge needle. Marrow injections were made via the tail vein 4 to 14 hours after the last injection of thio-TEPA. One-half the group served as untreated controls. Streptomycin 5.0 mg. in 0.5 ml. saline solution was administered subcutaneously daily for 12 days in some groups without improvement in survival, and was therefore not given routinely.

Healthy mongrel dogs, weighing 9-12 Kg., were housed with food and water available ad lib. Marrow aspiration, storage and administration were carried out as described previously. Thio-TEPA was administered intravenously in saline solution to each of two dogs, one acting as a control for the animal receiving marrow. Penicillin, 2 million units, and streptomycin, 0.25 Gm., and Ringer's solution, approximately 55 ml./Kg., were given routinely for the first 5 days after administration of thio-TEPA. Counts of white cells and platelets were performed periodically until death or recovery of the animal.

RESULTS

Preliminary studies in the mouse given thio-TEPA intravenously as a single dose revealed an LD₅₀ 30-day dose of 0.5 mg. (20 mg./Kg.) and an

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†Difco Laboratories, Detroit, Mich.
Table 1.—Effect of Infusions of Isologous Marrow on Survival Rates in BDF$_1$ Mice Given Intravenous Injections of thio-TEPA

<table>
<thead>
<tr>
<th>Thio TEPA 1.V.</th>
<th>ISOLOGOUS BONE MARROW</th>
<th>SURVIVED/TREATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7 mgm.</td>
<td>None 8-12 x 10$^6$</td>
<td>9/42</td>
</tr>
<tr>
<td>0.9 mgm.</td>
<td>None 8-20 x 10$^6$</td>
<td>5/25</td>
</tr>
<tr>
<td>1.0 mgm.</td>
<td>None 10 x 10$^6$</td>
<td>0/17</td>
</tr>
<tr>
<td>0.5 mgm. x 2</td>
<td>None 8 x 10$^6$</td>
<td>0/17</td>
</tr>
</tbody>
</table>

Table 2.—Effect of Infusions of Isologous Marrow on Survival Rates of BDF$_1$ Mice Given thio-TEPA Intraperitoneally (Thio-TEPA Was Given 5 Days Each Week)

<table>
<thead>
<tr>
<th>Thio TEPA I.P.</th>
<th>DEAD BEFORE MARROW</th>
<th>BONE MARROW</th>
<th>SURVIVED/TREATED</th>
<th>TOTAL SURVIVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>.050 mgm.</td>
<td>$\frac{9}{20}$</td>
<td>None 10 x 10$^6$</td>
<td>$\frac{19}{10}$</td>
<td>$\frac{19}{10}$</td>
</tr>
<tr>
<td>.065 mgm.</td>
<td>$\frac{9}{20}$</td>
<td>None 10 x 10$^6$</td>
<td>$\frac{7}{10}$</td>
<td>$\frac{7}{10}$</td>
</tr>
<tr>
<td>.080 mgm.</td>
<td>$\frac{5}{22}$</td>
<td>None 10 x 10$^6$</td>
<td>$\frac{7}{8}$</td>
<td>$\frac{7}{11}$</td>
</tr>
<tr>
<td>.100 mgm.</td>
<td>$\frac{17}{27}$</td>
<td>None 10 x 10$^6$</td>
<td>$\frac{9}{5}$</td>
<td>$\frac{9}{14}$</td>
</tr>
<tr>
<td>.125 mgm.</td>
<td>$\frac{23}{27}$</td>
<td>None 10 x 10$^6$</td>
<td>$\frac{0}{2}$</td>
<td>$\frac{0}{13}$</td>
</tr>
</tbody>
</table>

LD$_{100}$ 30-day dose of 1.0 mg. (40 mg./Kg.). Initial attempts to modify the mortality rates were made at doses of 0.7 mg. (28 mg./Kg.) to 1.0 mg. (40/mg./Kg.) of thio-TEPA. The results of these studies are shown in table 1. In the group treated with bone marrow there was a slight decrease in mortality. The mean survival time of animals dying in each group was 5 days. No change in survival was obtained by dividing the total dose into two daily doses. Histologic sections at autopsy showed the marrow hypoplasia that is to be expected in animals dying within 5 days after thio-TEPA administration. There was also a sloughing of intestinal epithelium.
Table 2 shows the effect of infusions of bone marrow on the mortality of mice given thio-TEPA intraperitoneally five times weekly for 3 to 5 weeks. At doses of 0.05 to 0.065 mg., all the animals survived the period of treatment with thio-TEPA. Almost all recovered uneventfully whether given marrow or not. At doses of 0.08 mg., about one-fourth of the animals died during thio-TEPA administration. Survival rates were the same whether marrow was given or not. At doses of 0.10 to 0.125 mg., mortality was so severe that over-all survival, even in the group treated with marrow, was not impressive.

Figure 1 and 2 show the peripheral white blood cell and platelet counts of six dogs given a single injection of 5.0 mg./Kg. of thio-TEPA intravenously. The dotted line gives the average counts and the variation within the group for the three dogs that were given thio-TEPA followed only by supportive treatment. The solid line gives the average counts and the variation within the group for the three dogs that were given thio-TEPA followed in 24 hours by an infusion of autologous marrow that had been stored for 3 to 4 weeks at −80 C. in 10 per cent dimethyl-sulphoxide.8 The three dogs that were given infusions of autologous marrow all showed prompt restoration of marrow function and survived. The control animals all remained aplastic and died.

The data of table 3 suggest that in the dog, as in the mouse, the usefulness of infusions of marrow falls off at higher doses of thio-TEPA where intestinal complications become more severe.

**DISCUSSION**

The results presented indicate that isogeneic marrow in the mouse does not provide a useful protection against the lethal effect of thio-TEPA in the doses and dose schedules studied. Attempts to produce a selective depression...
Table 3.—Effect of Infusions of Stored Autologous Marrow on Survival Rates of Dogs Given Single Intravenous Injections of thio-TEPA at Several Dose Levels

<table>
<thead>
<tr>
<th>Thio-TEPA mg/kg, iv.</th>
<th>AUTOLOGOUS MARROW INFUSION</th>
<th>30 DAY SURVIVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>NO</td>
<td>3/3</td>
</tr>
<tr>
<td>4.0</td>
<td>NO</td>
<td>2/4</td>
</tr>
<tr>
<td>5.0</td>
<td>YES</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>0/3</td>
</tr>
<tr>
<td>6.0</td>
<td>YES</td>
<td>2/3</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>0/3</td>
</tr>
<tr>
<td>8.0</td>
<td>YES</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>0/3</td>
</tr>
</tbody>
</table>

of marrow by the chronic administration of thio-TEPA and to lessen mortality from intestinal complications by the use of streptomycin were not successful. In view of the early time of death and sloughing of intestinal epithelium observed, mortality from this agent apparently involves organs other than bone marrow. These results are in contrast to those obtained after lethal x-irradiation (850 r) where an infusion of marrow leads to a survival of 95 per cent.10

In the dog, infusion of stored autologous marrow proved to be life-saving in the range of 5.0 mg./Kg. to 6.0 mg./Kg. of thio-TEPA. Below 4.0 mg./Kg., all dogs lived regardless of whether or not they received autologous marrow; above 6.0 mg./Kg., all dogs died.

Our results, although limited to thio-TEPA, indicate that for the effective use of stored autologous marrow in cancer chemotherapy in man, an assessment of marrow toxicity versus tumor toxicity is required for each drug and for each dose and dose schedule. In the case of drugs that significantly affect organs other than marrow, infusions of marrow are not likely to permit the administration of greatly increased doses.

SUMMARY

In mice and in dogs, the usefulness of infusions of marrow in reducing the toxicity of thio-TEPA is limited to a very narrow range of dose of drug in which prompt restoration of hematopoiesis seems critical to survival. Below this range, control animals recover spontaneously; above this range, damage to other organ systems precludes survival.

SUMMARIO IN INTERLINGUA

In muses e canes, le utilitate de infusiones de medulla in reducer le toxicitate de thio-TEPA es restringite a un multo restringite area de doses del pharmaco,
intra le qual le prompte restauration del hematopoiese pare esser de importanza critic pro le superviventia del animal. Infra iste area de valores, le animales de controlo se restabili spontaneemente (sin reciper medulla). Supra illo, le damno in altere systemas de organos prohibi le superviventia.

REFERENCES

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