Recovery of Lethally Irradiated Dogs Following Infusion of Autologous Marrow Stored at Low Temperature in Dimethyl-Sulphoxide

By John A. Cavins, Shinpei Kasakura, E. Donnall Thomas and Joseph W. Ferreebee

Dogs lethally exposed to general radiation usually make uneventful recoveries if given prompt infusions of a few billion autologous marrow cells. Cells for these infusions can be preserved indefinitely at low temperature in glycerol. Recent studies suggest that dimethyl-sulphoxide may be preferable to glycerol for these preservations. This communication reports the survival of dogs given 1200 r and infusions of autologous marrow stored at −80 C. in 10 per cent dimethyl-sulphoxide.

Materials and Methods

Sixteen dogs were used as experimental animals: thirteen beagles and three mongrels. The dogs were between 5 and 12 months of age and weighed from 10 to 30 pounds. Marrow was obtained by aspiration through a surgical window in the femora and humeri. Suspensions of cells in tissue culture fluid with added plasma were prepared as previously described. The suspensions were centrifuged for 10 minutes at 1000 rpm in an International PR-2 centrifuge at room temperature. The upper layer of fat was removed and discarded, and a portion of the supernatant fluid was poured off and used to prepare dilutions of dimethyl-sulphoxide. An equal quantity of 10, 20 or 30 per cent dimethyl-sulphoxide so prepared was then added to the marrow sediment and fluid remaining in the centrifuge tube to make a final suspension of marrow cells in a dimethyl-sulphoxide concentration of 5, 10 or 15 per cent. This suspension, 50 to 100 ml., was transferred to a 600-ml. plastic bag and frozen in a ¼ cm. layer between copper plates. The freezing rate was approximately 1 C. per minute to −25 C. and somewhat faster to final storage at −80 C.

Several weeks after their marrow had been stored, the dogs were irradiated with 1200 r from dual Co sources at a dose rate of 5 to 6 r per minute. The LD100 dose of this radiation in this laboratory is 600 r. Care before and after radiation was given the dogs as described previously.

When dog marrow has been frozen in hypertonic, 15 per cent glycerol, DNA slime in troublesome amount is apt to appear during the stepwise dilution that is necessary for the adjustment of the intracellular toxicity of the cells prior to their intravenous administration. In preliminary experiments it was found that no similar adjustment of toxicity is required with cells of dog marrow frozen in 10 per cent dimethyl-sulphoxide. After they have been thawed, these cells may be poured directly into large volumes of isotonic saline without rupture or formation of DNA slime. In the experiments here reported, the marrow was administered intravenously to the donor of origin without further treatment immediately after it had been thawed by brief and brisk agitation in a 37 C. water bath.

From the Mary Imogene Bassett Hospital (affiliated with Columbia University) Cooperstown, N. Y.


Submitted May 29, 1962; accepted for publication Aug. 21, 1962.

*Fenwal Laboratories, Morton Grove, Illinois.

730

Blood, Vol. 20, No. 6 (December), 1962
RECOVERY OF LETHALLY IRRADIATED DOGS

Table 1.—Summary of Experiments in Storing Marrow in Dimethyl-Sulphoxide

<table>
<thead>
<tr>
<th>Dog</th>
<th>No. of Marrow Cells Stored (x 10^9)</th>
<th>Dimethyl-Sulphoxide (per cent)</th>
<th>Storage Time (days)</th>
<th>Cell Loss* (per cent)</th>
<th>Marrow &quot;Take&quot;</th>
<th>Clinical Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>603</td>
<td>2.50</td>
<td>10</td>
<td>19</td>
<td>0</td>
<td>yes</td>
<td>pancreatic insufficiency</td>
</tr>
<tr>
<td>602</td>
<td>2.40</td>
<td>10</td>
<td>15</td>
<td>11</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>681</td>
<td>2.50</td>
<td>10</td>
<td>8</td>
<td>26</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>687</td>
<td>1.6</td>
<td>10</td>
<td>22</td>
<td>20</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>688</td>
<td>1.75</td>
<td>10</td>
<td>14</td>
<td>16</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>753</td>
<td>2.00</td>
<td>10</td>
<td>16</td>
<td>9</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>721</td>
<td>5.2</td>
<td>10</td>
<td>30</td>
<td>—</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>724</td>
<td>1.80</td>
<td>10</td>
<td>14</td>
<td>—</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>722</td>
<td>3.4</td>
<td>10</td>
<td>10</td>
<td>—</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>606</td>
<td>1.1</td>
<td>5</td>
<td>18</td>
<td>—</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>752</td>
<td>1.1</td>
<td>5</td>
<td>13</td>
<td>26</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>741</td>
<td>1.11</td>
<td>5</td>
<td>9</td>
<td>—</td>
<td>no</td>
<td>expired day 7</td>
</tr>
<tr>
<td>731</td>
<td>3.11</td>
<td>15</td>
<td>13</td>
<td>18</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>742</td>
<td>1.45</td>
<td>15</td>
<td>9</td>
<td>—</td>
<td>no</td>
<td>expired day 8</td>
</tr>
<tr>
<td>740</td>
<td>1.11</td>
<td>15</td>
<td>8</td>
<td>—</td>
<td>no</td>
<td>expired day 10</td>
</tr>
</tbody>
</table>

*This difference in cell counts before and after storage is presumably related to a tendency of the frozen and thawed cells to clump and thereby reduce the accuracy of the aliquots selected for counting.

Counts of nucleated cells were made on aliquots of the suspensions of marrow before they were frozen and on the stored specimens shortly after they had been thawed. Just as with cells preserved in glycerol, marrow cells preserved in dimethyl-sulphoxide are easily destroyed by the acetic acid of the fluids usually used in counting white cells. Counts were therefore made with a phase microscope with 5 per cent bovine albumin in Hank's solution as diluent, as previously described.3

RESULTS

Table 1 summarizes the results. The amount of marrow administered was from 1 to 5 billion nucleated cells. Cell counts after storage were between 74 and 100 per cent of the counts made before storage. The difference is presumably related to a tendency of the frozen and thawed cells to clump and to render inaccurate the aliquots selected for counting. Phase microscopy of wet film preparations of the thawed specimens revealed no other abnormality.

Nine dogs received marrow preserved in 10 per cent dimethyl-sulphoxide. Eight survived and did well. One, 603, made a normal recovery but died of chronic exocrine pancreatic insufficiency 5 months postirradiation. Figures 1 and 2 show the hematologic responses of several of these dogs. Restoration of hematopoiesis was prompt and complete in each.

Three dogs were given marrow preserved in 5 per cent dimethyl-sulphoxide. One died. Three were given marrow preserved in 15 per cent. Two died. Deaths were associated with slow restoration of hematopoiesis.

DISCUSSION

The preliminary studies reported here show that lethally irradiated dogs recover following the intravenous administration of samples of autologous
Fig. 1.—Peripheral white blood cell counts of dogs exposed to 1200 r Co60 radiation and the following day given an infusion of a sample of autologous marrow that had been stored at −80 C. in 10 per cent dimethyl-sulphoxide.

Fig. 2.—Peripheral blood platelet counts of dogs exposed to 1200 r Co60 radiation and the following day given an infusion of a sample of autologous marrow that had been stored at −80 C. in 10 per cent dimethyl-sulphoxide. In conformity with other work, storage in 5 and 15 per cent dimethyl-sulphoxide appeared less good.

A practical advantage of dimethyl-sulphoxide over glycerol is its more rapid exchange across the cell membranes of nucleated cells. The speed of this exchange permits intravenous infusion of cells stored in 10 per cent concentrations of this compound without adjustment of toxicity prior to admin-
istra tion. The adjustments by serial dilution with isotonic media that are required in the case of glycerol are frequently accompanied by the formation of a troublesome amount of DNA slime. With rapid thawing and immediate administration, slime formation has not been a problem with samples of dog marrow frozen in dimethyl-sulphoxide. The cell loss reported during storage is an estimate that is high because of loss that occurs during the shaking and counting of the fragile thawed specimens.

In the quantities used here—50 to 100 ml. of 5 to 15 per cent solution—dimethyl-sulphoxide showed no recognized toxicity. A disagreeable odor emanated from the animals a few minutes after their intravenous infusions. The odor was not noticed 24 hours later. The occurrence of chronic exocrine pancreatic insufficiency in one animal is not to be attributed to the infusion of dimethyl-sulphoxide. A similar complication has been seen in irradiated dogs given infusions of fresh or glycerol-frozen marrow.

SUMMARY

Sixteen normal young dogs were exposed to 1200 r of Co\textsuperscript{60} radiation over their entire bodies continuously at 5 to 6 r per minute. They were then given intravenous infusions of 1 to 5 billion of their own nucleated marrow cells that had been previously aspirated and stored frozen at \(-80\) C. in dimethyl-sulphoxide.

Nine received samples stored in 10 per cent dimethyl-sulphoxide. All made prompt clinical and hematologic recoveries. Three dogs received samples stored in 5 per cent dimethyl-sulphoxide. One died. Three received samples stored in 15 per cent dimethyl-sulphoxide. Two died.

No toxicity of dimethyl-sulphoxide was recognized in these experiments. A disagreeable odor emanated from the injected animals and persisted for a number of hours.

Dimethyl-sulphoxide in 10 per cent concentration is suitable for the preservation of dog marrow at low temperature. Because the specimens preserved in it can be administered intravenously without prior dilution, this additive has an advantage over glycerol.

SUMMARIO IN INTERLINGUA

Dece-sex normal juvene canes esseva exponite a 1200 r de irradiation del corpore total ab un fonte de Co\textsuperscript{60} in un sol application al rhythmo de 5 a 6 r per minuta. Postea illos recipeva 1 a 5 milliardos de lor proprie nucleate cellulas medullari que previemente habeva essite aspirate e magasinate a \(-80\) C in sulfoxydo dimethylic.


Nulle toxicitate de sulfoxydo dimethylic esseva recognoscite in iste experimentos. Un disagradabile odor emanava ab le animales post le injection, persistente durante un numero de horas.
Sulfoxydo dimethylic in un concentration de 10 pro cento es utile pro preservar medulla canin a basse temperaturas. Viste que le specimen preser-vate in illo pote esser administrate sin dilution, iste medio ha un avantage in comparation con glycerol.

REFERENCES


John A. Cavins, M.D., Research Associate, Mary Imogene Bassett Hospital, Cooperstown, N. Y.

Shinpei Kasakura, M.D., Research Fellow, Mary Imogene Bassett Hospital, Cooperstown, N. Y. (Supported by Cancer Research Training Grant CRT-5087 from the United States Public Health Service.)

E. Donnall Thomas, M.D., Physician-in Chief, Mary Imogene Bassett Hospital, Cooperstown, N. Y.; Associate Clinical Professor of Medicine, Columbia University, New York, N. Y.

Joseph W. Ferrebee, M.D., Research Physician, Mary Imogene Bassett Hospital, Cooperstown, N. Y.; Associate Clinical Professor of Medicine, Columbia University, New York, N. Y.
Recovery of Lethally Irradiated Dogs Following Infusion of Autologous Marrow Stored at Low Temperature in Dimethyl-Sulphoxide

JOHN A. CAVINS, SHINPEI KASAKURA, E. DONNALL THOMAS and JOSEPH W. FERREBEE