Autologous Bone Marrow Grafts in Dogs Treated with Lethal Doses of Cyclophosphamide

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SUMMARY

Dogs were given a single intravenous injection of 100 mg of cyclophosphamide per kilogram of body weight. Ten dogs received supportive therapy only and died within 14 days with profound marrow hypoplasia. Ten dogs were given stored autologous marrow 24 hours after administration of cyclophosphamide. Eight showed prompt hemopoietic recovery and survived. It was concluded that stored autologous bone marrow is effective in restoring hemopoietic function following an otherwise lethal dose of cyclophosphamide.

INTRODUCTION

One of the limiting factors in the use of cytostatic drugs is their hemopoietic toxicity. A number of attempts has been made to increase the tolerable dose of these drugs by protecting the organism from prolonged marrow depression, with the infusion of autologous hemopoietic tissue obtained prior to drug administration. Such work in man has centered on the treatment of malignancies in which bone marrow involvement could not be identified (3, 5, 9, 10, 11). The rationale for this approach is based on rodent experiments demonstrating protection from otherwise lethal doses of chemotherapeutic agents by isogeneic bone marrow (4, 6, 12, 13, 15). Only limited information is available in larger mammals and in man suggesting beneficial effects from autologous marrow (3, 5, 6, 9, 11). The present study was undertaken to evaluate the effect of stored autologous bone marrow on hemopoietic recovery and survival of dogs given lethal doses of cyclophosphamide.

MATERIALS AND METHODS

Twenty-four dogs, 6 to 12 months of age and weighing 6 to 20 kg, were isolated and observed for at least 21 days prior to use. They were dewormed and immunized against hepatitis and distemper. All dogs were given single dose of cyclophosphamide (Mead Johnson and Company, Evansville, Indiana), 100 mg/kg of body weight. The cyclophosphamide was added to an intravenous infusion of Ringer's solution and administered over a period of one hour. A total of 40 ml of Ringer's solution per kg was infused to assure adequate initial hydration. Parenteral fluid administration was continued in amounts of 150 to 250 ml/kg per day for the first 6 days because of the severe gastroenteritis encountered. Sodium ampicillin, 250 mg, was injected twice daily during the period of leukopenia. White blood counts, platelet counts, and hematocrits were performed at least three times a week.

Bone marrow for autologous marrow grafts was prepared from aspirates of both femora by the method of Mannick et al. (7). Approximately 50 ml of marrow suspended in TC 199 tissue culture medium (Difco Lab, Detroit, Michigan) was placed in each of two 600-ml plastic blood administration bags (Fenwal Lab, Morton Grove, Illinois). An equal volume of a mixture of 70% TC 199, 20% dimethyl sulfoxide, and 10% autologous serum was then added to the bags (2). The marrow suspension was frozen to −25°C at a rate of 1°per minute (16) and stored at −80°C for 15 to 29 days. Twenty-four hours after the administration of cyclophosphamide, the marrow was thawed rapidly in a 37°C water bath and infused. In 4 dogs, autologous bone marrow was obtained immediately prior to cyclophosphamide administration and kept at 4°C in TC 199 medium containing 10—20% canine plasma. The marrow was infused either 4 or 8 hours after cyclophosphamide. Dogs in marrow-treated and untreated groups were evaluated for survival and hemopoietic recovery.

RESULTS

Ten dogs given cyclophosphamide but no bone marrow died within 14 days (Table 1). Cyclophosphamide in the dose given produced a profound leukopenia in 4 to 6 days which persisted (Chart 1). Marrow examination at autopsy in 7 of these dogs lying between Days 5 and 12 showed severe hypoplasia in 4 instances and early regeneration in 3.

Ten dogs were given stored autologous marrow 24 hours after cyclophosphamide. Two of these 10 animals died on Days 5 and 7 with low white blood counts and severe marrow hypoplasia at autopsy. Eight dogs survived for more than 30 days and were clinically well when sacrificed (Table 1). They showed rapid recovery of the white blood counts beginning by Day 7 following cyclophosphamide (Chart 2). Platelet counts
Table 1

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>No. of marrow cells infused × 10^6</th>
<th>Marrow storage temperature (°C)</th>
<th>Marrow storage time</th>
<th>Time (hr) of marrow infusion after cyclophosphamide</th>
<th>Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>None</td>
<td>-80</td>
<td>15 days</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
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<td>15 days</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
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<td>-80</td>
<td>29 days</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
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<td>-80</td>
<td>29 days</td>
<td>24</td>
<td>5</td>
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<td>-80</td>
<td>16 days</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
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<td>5 hr</td>
<td>4</td>
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</tr>
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<td>5 hr</td>
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<td>8</td>
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<tr>
<td>345</td>
<td>None</td>
<td>+4</td>
<td>9 hr</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

Survival of dogs with and without autologous bone marrow infusions following cyclophosphamide.

fell to values below 5,000 per cu mm by the 9th or 10th day and returned to normal levels 3 to 4 days after the recovery of the white blood count.

Four dogs were given autologous marrow which had been stored at 4°C. Two of these dogs received the infusion 4 hours after cyclophosphamide and 2 were given marrow 8 hours after the drug (Table 1). All four dogs died by Day 8 without evidence of marrow recovery (Chart 3).

Vomiting, diarrhea and hematuria were noted in addition to marrow toxicity. Vomiting began within hours following cyclophosphamide and tended to subside over the subsequent 6 days. Diarrhea began approximately three days after cyclophosphamide and lasted until the end of the first week in surviving animals. The diarrhea was severe, and daily parenteral support with electrolyte solutions was required. The hematuria occurred despite efforts to maintain hydration and diuresis. Hematuria was observed from 2 to 15 days following cyclophosphamide. Dogs that died showed evidence of hemorrhagic cystitis at autopsy. In addition, the 11 dogs that were autopsied showed hemorrhagic pneumonia and variable degrees of hemorrhagic gastroenteritis particularly involving the distal duodenum and upper jejunum.

**DISCUSSION**

The present studies show that dogs can be protected from the lethal hemopoietic toxicity of cyclophosphamide by the administration of stored autologous bone marrow. The protective effect of isogeneic bone marrow infusions in rodents has been demonstrated following lethal doses of a number of cytotoxic drugs, including cyclophosphamide (13), Myleran (4, 19), triethylenemelamine (12), nitrogen mustard (18), thioguanine (15) and triethylenethiophosphoramide (Thio-TEPA) (6). In larger mammals and in man, the protective effect of stored autologous marrow has been more difficult to demonstrate. Lochte et al. (6) evaluated this question with Thio-TEPA in dogs and were able to show protection in the 5 to 6 mg/kg range but no protection at 8 mg/kg. In dogs receiving nitrogen mustard, death occurred despite the infusion of stored autologous marrow (17). In contrast, dogs can be protected against 2–3 times the lethal dose of whole body irradiation by autologous marrow (7). In man, a number of studies using alkylating agents, primarily nitrogen mustard, have suggested some beneficial effect from autologous marrow infusions (3, 5, 9, 11). The doses of nitrogen mustard used in the clinical studies for the most part were in the range where spontaneous marrow recovery might be expected to occur.

In the present study, the stored bone marrow was infused 24 hours following cyclophosphamide administration. This was done in order to avoid the destruction of the infused marrow cells by persisting cyclophosphamide activity (1). Santos et al. (14), however, using the adoptive transfer system in mice have reported that the immunosuppressive activity of the drug was no longer effective after 60 minutes. In the 4 dogs receiving marrow within 8 hours of cyclophosphamide administration, hemopoietic recovery failed to occur, suggesting significant residual levels of cyclophosphamide at these times. Studies by Mannick et al. have demonstrated the viability of marrow stored at 4°C for up to 24 hours (8).
Chart 1. White blood cell changes in 10 dogs receiving 100 mg cyclophosphamide per kg and no bone marrow infusions.

Chart 2. White blood cell changes in 10 dogs receiving 100 mg cyclophosphamide per kg. Stored autologous bone marrow was infused 24 hours after cyclophosphamide.

Chart 3. White blood cell changes in 4 dogs receiving 100 mg cyclophosphamide per kg. Autologous bone marrow was infused either 4 or 8 hours after cyclophosphamide.

Cyclophosphamide toxicity involving the gastrointestinal and urinary tracts was severe at the dose level used in these dogs. With increasing doses these nonhemopoietic toxic effects may limit the usefulness of the autologous marrow. The dose range of cyclophosphamide in which infusions of autologous bone marrow can prevent death remains to be defined for the dog.

REFERENCES


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