Busulfan and Total Body Irradiation as Antihematopoietic Stem Cell Agents in the Preparation of Patients With Congenital Bone Marrow Disorders for Allogeneic Bone Marrow Transplantation

By Robertson Parkman, Joel M. Rappeport, Samuel Hellman, Jeffrey Lipton, Brian Smith, Raif Geha, and David G. Nathan

The capacity of busulfan and total body irradiation to ablate hematopoietic stem cells as preparation for the allogeneic bone marrow transplantation of patients with congenital bone marrow disorders was studied. Fourteen patients received 18 transplants; busulfan was used in the preparatory regimen of eight transplants and total body irradiation in the regimens of six transplants. Sustained hematopoietic ablation was achieved in six of eight patients prepared with busulfan and in all six patients prepared with total body irradiation. Three patients prepared with total body irradiation died with idiopathic interstitial pneumonitis, whereas no patients receiving busulfan developed interstitial pneumonitis. The optimal antihematopoietic stem cell agent to be used for the preparation of patients with congenital bone marrow disorder for bone marrow transplantation is not certain.

CONGENITAL DISORDERS of bone marrow function involve cells derived from either the lymphoid and/or hematopoietic stem cells. Allogeneic bone marrow transplantation has been demonstrated to be an efficacious form of therapy for diseases such as the Wiskott-Aldrich syndrome, severe combined immune deficiency, thalassemia, infantile agranulocytosis, etc, if preparative regimens that ablate the immune deficiency, thalassemia, infantile agranulocytosis, etc, if preparative regimens that ablate the abnormal stem cells are used.1-11 We initially reported the use of total body irradiation (TBI) as an antihematopoietic stem cell agent in the pretransplant preparatory regimen. Recently, others have reported the use of busulfan (BUS) or dimethyl busulfan as alternative antihematopoietic stem cell agents to avoid the toxicities associated with the use of total body irradiation.9-13 We report our experience in the transplantation of 14 patients with congenital bone marrow disorders. The patients received 18 transplants; busulfan was used as the antihematopoietic stem cell agent in eight cases and total body irradiation in six cases. The relative effectiveness and toxicity of the two antihematopoietic stem cell agents are described.

PATIENT SELECTION AND TRANSPLANT PREPARATION

Fourteen patients with potentially fatal congenital disorders of bone marrow function were considered for bone marrow transplantation. Patients were transplanted from HLA-A, -B, and -D identical donors if available. If no HLA-identical donor was available, a partially histocompatible donor was used. When a haploidentical parental donor was used (UN 099), the bone marrow was treated in vitro with a monoclonal antibody to mature T lymphocytes (T-12) and complement, as previously described.4 HLA typing was performed by standard lymphocytotoxicity testing and mixed lymphocyte culture methods.13-16 Red blood cell antigens were determined by the Center for Blood Research, Boston. All patients were transplanted under protocols approved by the Committee on Human Investigations.

Patients were maintained in Laminar air flow or positive pressure isolation and received oral antibiotics, antibacterial skin care, and a low bacteria diet, as previously described.17 Patients were isolated from day 10 to discharge, approximately days 50 to 60.

Patients were prepared for eight transplants with the busulfan protocol (BUS), consisting of rabbit anti-human thymocyte serum (ATS), 0.2 mL/kg, IV, on days -9 and -7; cyclophosphamide, 50 mg/kg, IV, on days -5, -4, -3, and -2; and busulfan, 2 mg/kg, PO, on days -9, -8, -7, and -6. For six transplants, the total body irradiation protocol (TBI) consisted of procarbazine, 12.5 mg/kg, PO, on days -8, -6, and -4; ATS, 0.2 mL/kg, IV, on days -7 and -5, and total body irradiation, 750 to 900 R midline dose at 5 rad/min, on day -1 from a 4-MEV linear accelerator.18 Preparation for four transplants contained neither busulfan nor total body irradiation: one patient (UN 043) received cytosine arabinoside, 150 mg/m², daily by continuous IV infusion from days -16 to -10, and cyclophosphamide, 50 mg/kg, IV, on days -5, -4, -3, and -2; one patient (UN 020) received ATS, 0.2 mL/kg, IV, on days -7, -5, and -3; procarbazine, 12.5 mg/kg, PO, on days -6, -4, and -2, and cyclophosphamide, 50 mg/kg, IV, on days -5, -4, -3, and -2; and one patient with severe combined immune deficiency (UN 099) received two transplants: no preparation for her first transplant, and ATS, 0.2 mL/kg, on days -5 and -3, and cyclophosphamide, 50 mg/kg, IV, on days -5, -4, -3, and -2 for her second transplant. Patients were transplanted on day 0, with bone marrow cell doses varying from 1.0 to 5.9 x 10⁸ nucleated bone marrow cells per kg. Hematopoietic and lymphoid engraftment was documented by a change in the karyotype of spontaneously dividing...
bone marrow cells, red blood cell antigens, the karyotype of phytohemagglutinin (PHA) stimulated peripheral blood lymphocytes, and/or HLA typing.

**RESULTS**

**Patient Population**

Fourteen patients with congenital disorders of their hematopoietic and/or lymphoid stem cells have been transplanted at Children's Hospital Medical Center (Table 1). Six of the patients had the Wiskott-Aldrich syndrome; four had granulocyte disorders, including primary actin deficiency, infantile agranulocytosis, and chronic granulomatous disease; two patients had severe combined immune deficiency, and one patient each had Gaucher's disease and an immunodeficiency due to GPL-115 deficiency. The patients ranged in age from 2 months to 15 years, and the male to female ratio was 1:2:2. Five patients had defects restricted to cells derived from their hematopoietic stem cells, two to cells derived from their lymphoid stem cells, and seven had defects that were expressed in both lymphoid- and hematopoietic-derived cells (six patients with the Wiskott-Aldrich syndrome and one patient with severe combined immune deficiency and agranulocytosis).

**Genetic Relationship of the Donors and Recipients**

Of the eight patients who received busulfan in their preparatory regimen, four had HLA-identical sibling donors; one had an HLA-B and -D identical but HLA-A nonidentical sibling donor due to an A-B crossover; one had an HLA-identical paternal donor; one had a D locus-identical parental donor; and one had a haploidentical parental donor whose bone marrow was treated in vitro with monoclonal T-12 anti-body and complement (Table 2). The six patients who received total body irradiation all had HLA-identical sibling donors (Table 3).

**Busulfan Regimen**

Eight patients were prepared for transplantation with busulfan as an antihematopoietic stem cell agent and rabbit anti-human thymocyte serum ± cyclophosphamide as immunosuppressive agents. No change in the clinical condition of the patients was observed following the busulfan administration; there were no fluid shifts, fever, or severe diarrhea. In all cases, hematopoietic ablation and donor hematopoietic engraftment was achieved (Table 2). Complete donor hematopoietic engraftment was not sustained in two patients. After initial evidence of both donor hematopoietic and lymphoid engraftment, one patient with severe combined immune deficiency and agranulocytosis (UN 105) had a reversion to lymphoid and hematopoietic cells of recipient origin. Five months following her transplant, 90% of her spontaneously dividing bone marrow cells were of donor origin by karyotype analysis (XY), while nine months following transplantation, all spontaneously dividing bone marrow cells and all PHA-stimulatable peripheral lymphocytes were of recipient origin (XX). Another patient with severe combined immune deficiency (UN 099) has sustained split hematopoietic chimerism. Fifteen months following transplantation, 75% of the patient's purified monocytes are of recipient origin, as determined by HLA typing, whereas all her erythrocytes, lymphocytes, and granulocytes are of donor origin, as determined by red blood cell antigens and HLA typing. Thus, sustained hematopoietic ablation and complete donor hematopoietic ablation and donor hematopoietic engraftment was achieved (Table 2). Complete donor hematopoietic engraftment was not sustained in two patients. 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Table 2. Patients Transplanted for Congenital Disorders With Busulfan

<table>
<thead>
<tr>
<th>Unique Patient Number</th>
<th>Donor Preparation</th>
<th>Hematopoietic Ablation</th>
<th>Engraftment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>099 Mother—haploidentical</td>
<td>(1) None* (2) ATS, CTX† (3) ATS, CTX, BUS</td>
<td>No (No) Partial</td>
<td>No engraftment (No engraftment) Hematopoietic and lymphoid engraftment</td>
<td>Repeat transplant (Repeat transplant) Alive and well, 42 mo; monocytes of recipient origin</td>
</tr>
<tr>
<td>100 Sibling—histocompatible</td>
<td>ATS, CTX, BUS</td>
<td>Yes</td>
<td>Hematopoietic and lymphoid engraftment</td>
<td>Alive and well, 48 mo</td>
</tr>
<tr>
<td>103 Sibling—histocompatible</td>
<td>ATS, CTX, BUS</td>
<td>Yes</td>
<td>Hematopoietic and lymphoid engraftment</td>
<td>Alive and well, 45 mo</td>
</tr>
<tr>
<td>105 Father—D locus-identical</td>
<td>ATS, CTX, BUS</td>
<td>Temporary</td>
<td>Hematopoietic and lymphoid engraftment; reversion to autologous cells at 9 mo</td>
<td>Alive and well, 30 mo; all cells of recipient origin</td>
</tr>
<tr>
<td>107 Sibling—HLA-B and -D compatible, A/B crossover</td>
<td>ATS, CTX, BUS</td>
<td>Yes</td>
<td>Hematopoietic and lymphoid engraftment</td>
<td>Died, day 35; sepsis</td>
</tr>
<tr>
<td>112 Sibling—histocompatible</td>
<td>ATS, CTX, BUS</td>
<td>Yes</td>
<td>Hematopoietic and lymphoid engraftment</td>
<td>Died, 13 mo; E. coli sepsis</td>
</tr>
<tr>
<td>121 Sibling—histocompatible</td>
<td>ATS, CTX, BUS‡</td>
<td>Yes</td>
<td>Hematopoietic and lymphoid engraftment</td>
<td>Alive, 22 mo; chronic graft-versus-host disease</td>
</tr>
<tr>
<td>139 Father—histocompatible</td>
<td>ATS, CTX, BUS‡</td>
<td>Yes</td>
<td>Hematopoietic and lymphoid engraftment</td>
<td>Alive and well, 12 mo</td>
</tr>
</tbody>
</table>

* Some patients received more than one transplant.
† ATS, rabbit anti-human thymocyte serum; CTX, cyclophosphamide; BUS, busulfan; 2 mg/kg x four days.
‡ Busulfan, 3 mg/kg x four days instead of 2 mg/kg x four days.

Hematopoietic engraftment was achieved in only six of eight patients. No interstitial pneumonitis or cataracts have been detected in the eight BUS patients.

Total Body Irradiation Regimen

Total body irradiation was used as an antihematopoietic stem cell agent in six transplants with procarbazine and antithymocyte serum as immunosuppressive agents (Table 3). In all cases, complete donor lymphoid and hematopoietic engraftment was achieved. Three of the six patients who received total body irradiation developed idiopathic interstitial pneumonitis, which contributed to the deaths of all three patients. No viral agents were isolated from any of the

Table 3. Patients Transplanted for Congenital Disorders With Total Body Irradiation

<table>
<thead>
<tr>
<th>Unique Patient Number</th>
<th>Donor Preparation</th>
<th>TBI Dose*</th>
<th>Engraftment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>020 Sibling—histocompatible</td>
<td>(1) ATS, PC, CTX†</td>
<td>750</td>
<td>No engraftment (Hematopoietic and lymphoid engraftment)</td>
<td>Repeat transplant (Died, day 53; interstitial pneumonitis)</td>
</tr>
<tr>
<td>043 Sibling—histocompatible</td>
<td>(1) Ara-C, CTX</td>
<td>890</td>
<td>Only T lymphoid graft; reversion to recipient T lymphocytes at 5 mo Hematopoietic and lymphoid engraftment</td>
<td>Alive and well, 8 yr</td>
</tr>
<tr>
<td>053 Sibling—histocompatible</td>
<td>ATS, TBI</td>
<td>862</td>
<td>Hematopoietic and lymphoid engraftment</td>
<td>Alive and well, 7 yr</td>
</tr>
<tr>
<td>063 Sibling—histocompatible</td>
<td>ATS, PC, TBI</td>
<td>850</td>
<td>Hematopoietic and lymphoid engraftment</td>
<td>Alive and well, 6 yr</td>
</tr>
<tr>
<td>066 Sibling—histocompatible</td>
<td>ATS, PC, TBI</td>
<td>800</td>
<td>Hematopoietic and lymphoid engraftment</td>
<td>Died, 6 mo; interstitial pneumonitis and myocarditis</td>
</tr>
<tr>
<td>080 Sibling—histocompatible</td>
<td>ATS, PC, TBI</td>
<td>800</td>
<td>Hematopoietic and lymphoid engraftment</td>
<td>Died, 6 mo; interstitial pneumonitis, chronic graft-versus-host disease, renal failure</td>
</tr>
</tbody>
</table>

* TBI dose given as midline dose in rads.
† ATS, rabbit anti-human thymocyte serum; PC, procarbazine; CTX, cyclophosphamide; Ara-C, cytosine arabinoside; TBI, total body irradiation; BUS, busulfan.
patients’ autopsies. One patient (UN 043) developed bilateral cataracts that did not require surgery.

**DISCUSSION**

Allogeneic bone marrow transplantation has the potential to cure congenital disorders of both the lymphoid and hematopoietic stem cells. Essential to the success of bone marrow transplantation is the ablation of the abnormal stem cells to permit the engraftment of the normal donor lymphoid/hematopoietic stem cells. Initially, patients with hematopoietic stem cell disorders were successfully transplanted following preparation with total body irradiation. The toxicities of total body irradiation has caused investigators to evaluate the clinical use of busulfan and its analog, dimethyl busulfan, in the treatment of congenital disorders of bone marrow function. Clinically, busulfan has antihematopoietic stem cell activity, and recently, its successful utilization in the preparation of patients with acute myelogenous leukemia for bone marrow transplantation has been reported. However, in our experience, as well as that of others treating metabolic diseases, busulfan has not reproducibly achieved hematopoietic ablation at the dose of 2 mg/kg × four days. Two of our eight patients who received busulfan had the complete or partial recovery of recipient hematopoiesis. The Westminster Bone Marrow Team has reported their transplantation results following busulfan preparation (2 mg/kg × four days or 80 mg/m² × six days). Two of three patients transplanted for Hurler’s disease required a second transplant because of the spontaneous recovery of donor hematopoiesis. Following a second transplant with the same busulfan dose, donor hematopoietic engraftment was achieved. The recovery of recipient hematopoiesis indicated that recipient hematopoietic stem cells were not ablated by the initial preparation. Two patients with Sanfilippo B disease did not achieve hematopoietic engraftment following their first transplant but were engrafted following a second transplant with the same busulfan dose. A sixth patient with metachromatic leukodystrophy achieved hematopoietic engraftment after her first transplant. Thus, four of six patients required two transplants or a total of 4 mg/kg × four days of busulfan to achieve donor hematopoietic engraftment. These results, in addition to ours, suggest that busulfan at a dose of 2 mg/kg × four days is not adequate to achieve hematopoietic stem cell ablation in all individuals.

Because busulfan is activated in vivo, in vitro data on its effect on human hematopoietic stem cells is difficult to obtain; however, in vivo murine experiments suggest that the dose–response curve of hematopoietic stem cells to busulfan in the total dose range of 10 to 100 mg/kg is not steep. Studies in dogs have demonstrated an LD₉₀ of 7.5 mg/kg for dimethyl myleran when it is given as a single dose to animals not maintained in sterile environments or given granulocyte transfusions.

Busulfan has been used at a dose of 4 to 6 mg/kg × four days in the preparation of patients with acute myelogenous leukemia for histocompatible bone marrow transplantation, and dimethyl busulfan, given as a single intravenous dose, 5 mg/kg, has been used as the preparation of patients with thalassemia and the Wiskott-Aldrich syndrome.

Total body irradiation has reproducibly ablated recipient hematopoiesis in patients with leukemia when given at a midline dose of 800 to 1,000 rad at a rate of 5 rad/min. When patients with leukemia were prepared for transplantation with chemotherapy alone, which did not include busulfan, three of 13 patients had recovery of recipient hematopoiesis. In addition, hematopoietic engraftment was not achieved in patients with the Wiskott-Aldrich syndrome and chronic granulomatous disease when they were prepared with chemotherapy alone, which did not include busulfan. Thus, total body irradiation is an effective antihematopoietic stem cell agent at the doses used as preparation for leukemia and congenital disorders (800 to 1,000 rad).

Although none of the 11 patients transplanted with an HLA-identical donor recovered recipient hematopoiesis, two of three patients transplanted with partially histocompatible donors had complete or partial recovery of autologous hematopoiesis. Following total body irradiation, there are acute fluid shifts, fever, and diarrhea, which can be severe. More importantly, approximately 40% of recipients...
who receive histocompatible bone marrow transplants for leukemia following preparation with total body irradiation develop interstitial pneumonitis. Three of the six patients, who were transplanted for congenital disorders and who received total body irradiation, developed fatal interstitial pneumonitis. No interstitial pneumonitis was observed in patients receiving only busulfan. Other potential side effects of total body irradiation include cataracts and an increased incidence of secondary neoplasia. The cataracts, which develop following total body irradiation, have their onset at approximately two to three years posttransplantation. The patients who received busulfan are just approaching the time at which the detection of cataracts might be expected.

We have increased the dose of busulfan for congenital disorders to 3 mg/kg x four days in an attempt to decrease the incidence of hematopoietic ablation failure. However, the toxicity of busulfan may equal that of total body irradiation when biologically equivalent doses are used. Attempts to reduce the toxicity associated with total body irradiation have included fractionating the total body irradiation dose and/or shielding the lungs. Reduction of the total body dose to the lung to 600 rad or fractionation of the dose to 300 rad x four days both have reduced the incidence of interstitial pneumonitis. At present, the optimal antihematopoietic stem cell agent for the transplantation of patients with congenital bone marrow disorders is uncertain.

REFERENCES


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