FK506 (Tacrolimus) Monotherapy for Prevention of Graft-Versus-Host Disease After Histocompatible Sibling Allogeneic Bone Marrow Transplantation


FK506 (Tacrolimus) is an immunosuppressive drug that blocks the activation of antigen-specific T lymphocytes, a major component in the pathogenesis of graft-versus-host disease (GVHD). This study was designed to obtain first estimates of the safety and efficacy of FK506 monotherapy in the prevention of GVHD following HLA-identical sibling marrow transplantation. Additionally, a subset of patients was studied to define the pharmacokinetic profile of FK506. Twenty-seven adult patients with leukemia or myelodysplasia received FK506 starting the day before transplant at a dose of 0.04 mg/kg/d by continuous intravenous infusion. When clinically possible, FK506 was given orally in two divided doses starting at five times the daily intravenous dose. FK506 doses were adjusted to target a steady state or trough blood level between 10 to 30 ng/mL. These patients were followed for 6 months posttransplant. All patients had sustained marrow engraftment. Frequently noted adverse events included reversible renal dysfunction, diarrhea, fever, nausea, vomiting, and headache. Most patients required FK506 dose reductions associated with elevated serum creatinine. Two (7%) patients relapsed, one of whom died of the disease within the 6-month study period. A second patient died due to pulmonary mucor. Whole blood pharmacokinetic parameters indicated a half-life of 18.2 ± 12.1 hours; volume of distribution of 1.67 ± 1.02 L/kg; clearance of 71 ± 34 mL/h/kg; and bioavailability of 32 ± 24%. Eleven of 27 (41%) patients developed grade II to IV acute GVHD, including grade II and one grade III. Six of 24 (25%) evaluable patients developed chronic GVHD. These data indicate that FK506 monotherapy has activity in preventing GVHD. Further studies of FK506 with lower doses to improve tolerability and in combination with other immunosuppressants to augment efficacy are warranted. © 1996 by The American Society of Hematology.

IMMUNOSUPPRESSIVE therapy is required after allogeneic bone marrow transplantation (BMT) to prevent significant graft-versus-host disease (GVHD) and to establish sustained donor-recipient immune tolerance. Two large, prospective, randomized clinical trials comparing cyclosporin A (CSA) monotherapy to CSA plus methotrexate or methotrexate monotherapy reported grade II to IV acute GVHD rates of 54% and 33%, grade III to IV acute GVHD rates of 26% and 17%, and chronic GVHD rates of 58% and 50% in the CSA monotherapy groups. In a retrospective review of 833 adult recipients of cyclosporine monotherapy prophylaxis after HLA-identical BMT, the overall incidence of acute GVHD was 43 ± 3%. Additionally, other studies of cyclosporine monotherapy for GVHD prophylaxis have reported rates of grade II to IV acute GVHD ranging from 27% to 47% and chronic GVHD rates ranging from 30% to 73%. Although clinical studies have demonstrated that the combination of cyclosporine with methotrexate or cyclosporine with methotrexate and prednisone is more effective than monotherapy regimens for the prevention of acute GVHD, the development of new agents may permit a further reduction in the incidence or severity of GVHD or a reduction in the toxicity associated with posttransplant immunosuppression. FK506 did not impair marrow engraftment and was effective in the prevention of GVHD in preclinical studies of allogeneic marrow transplantation. In addition, FK506 prevented both graft rejection and GVHD in preclinical studies of limb and small-bowel allografting. In preliminary clinical trials, FK506 demonstrated efficacy in the prevention and treatment of acute GVHD and in reversing or stabilizing chronic GVHD following allogeneic marrow transplantation. FK506 has been effective in the prevention and treatment of graft rejection in solid organ transplantation. Based on these observations, we conducted a multicenter phase II study to evaluate the safety and efficacy of FK506 monotherapy for the prevention of GVHD following genotypically identical allogeneic BMT therapy for leukemia or myelodysplasia. In addition, a subset of patients was studied to define the pharmacokinetic profile of FK506 in this patient population.

MATERIALS AND METHODS

Patients. Adult patients undergoing allogeneic BMT therapy using genetically matched marrow donors for leukemia or myelodysplasia were enrolled in the study from four participating centers: the Sammons Cancer Center, Baylor University Medical Center (Dallas, TX); the University of Minnesota (Minneapolis, MN); Emory University (Atlanta, GA); and the Brigham and Women’s Hospital, Harvard University (Boston, MA). The protocol and consent forms were approved by each center’s Institutional Review Board for Human Investigation. Written informed consent was obtained from all patients. Patients were excluded from the study if they had a prestudy Karnofsky score < 80%, estimated creatinine clearance < 60 mL/min, total bilirubin above the upper limit of normal, SGOT or SGPT > 1.5 × upper limit of normal, PFC or FEV1 < 75% predicted, cardiac ejection fraction < 50%, or known hypersensitivity to Cremophor or related products. In addition, patients were...
Tabulation. In addition, laboratory studies, including a complete blood count and serum chemistries, were obtained routinely. Renal dysfunction was defined as a doubling of serum creatinine over baseline. All concomitant medications were recorded and were used to assess treatment requirements for hyperglycemia, hypertension, and GVHD.

**Pharmacokinetics.** In 12 patients, serial blood samples were obtained over 24 hours following a single intravenous dose of FK506 at 0.02 mg/kg infused over a 4-hour period to determine the terminal half-life, total body clearance, and volume of distribution of FK506. Blood samples were drawn before and at 1, 2, 4, 4.25, 4.5, 4.75, 5, 6, 7, 8, 10, 12, 16, and 24 hours subsequent to initiation of the intravenous infusion. In 11 of the 12 patients, serial blood samples were obtained over 12 hours following consistent oral dosing for 4 consecutive days to assess bioavailability. Blood samples were drawn before and at 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours after dosing.

**Grading of GVHD.** Acute and chronic GVHD were graded and staged by clinicians at the study centers using previously described criteria. Biopsies were obtained when indicated to corroborate the clinical diagnosis of GVHD, but were not required. **Statistical analysis.** The primary efficacy end point was to measure the incidence of moderate-to-severe acute GVHD (grade II to IV) and chronic GVHD within the 6-month study period. Results are reported for all patients enrolled in the study (intention-to-treat analysis). Patients were censored for GVHD end points at the time of relapse or death. Kaplan-Meier curves were generated for relapse-free survival and patient survival.

**RESULTS**

From April 1992 to September 1992, 27 adult patients were enrolled in this trial. Patient characteristics are shown in Table 1.

**Drug administration/blood levels.** The initial intravenous FK506 dose of 0.04 mg/kg per day was maintained through 7 days posttransplant. In the patients as a group, the mean whole blood levels of FK506 ranged from 21 ± 8 to 37 ± 38 ng/mL from day 1 through day 7 posttransplant. Beyond day 7, intravenous FK506 doses were lowered primarily due to the elevated serum creatinine to approximately 0.03 mg/kg/d resulting in pooled mean whole blood levels ranging from 23 ± 11 to 24 ± 9 ng/mL. The median duration on intravenous therapy was 26 (6 to 39) days. Subsequent oral adjusted FK506 doses, before taper, were between 0.12 ± 0.07 and 0.15 ± 0.07 mg/kg/d, with corresponding pooled mean whole blood levels ranging from 16 ± 10 to 23 ± 13 ng/mL.

**Adverse events.** Nephrotoxicity was the primary adverse event associated with FK506. Twenty-five (92.6%) patients had a doubling of their baseline serum creatinine levels during the 6-month study period, with a mean peak serum creatinine of 2.6 ± 0.6 mg/dL. Renal function improved when FK506 doses were reduced or temporarily held. The mean serum creatinine at the end of the 6-month study period was 1.4 ± 0.6 mg/dL, and FK506 was inadvertently not continued in one patient on day 31 due to an elevated serum creatinine of 2.2 mg/dL, and FK506 was inadvertently not resumed. This patient did not receive subsequent immunosuppressive therapy and did not develop acute or chronic GVHD. A second patient developed an elevated serum creatinine of 2.3 mg/dL on day 147, and FK506 was discontinued.

**Table 1. Patient Demographics and Disease Characteristics**

<table>
<thead>
<tr>
<th>Patient</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary diagnosis, N (%)</td>
<td></td>
</tr>
<tr>
<td>CML, 1st chronic phase</td>
<td>13 (48)</td>
</tr>
<tr>
<td>AML, 1st or 2nd complete remission</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Relapse</td>
<td>1 (4)</td>
</tr>
<tr>
<td>MDS</td>
<td>6 (22)</td>
</tr>
<tr>
<td>ALL, 1 complete remission</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>40</td>
</tr>
<tr>
<td>Range</td>
<td>18-53</td>
</tr>
<tr>
<td>Preparative regimen, N (%)</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide, TBI</td>
<td>9 (33)</td>
</tr>
<tr>
<td>Etoposide, cyclophosphamide, TBI</td>
<td>8 (30)</td>
</tr>
<tr>
<td>Busulfan, cyclophosphamide</td>
<td>8 (30)</td>
</tr>
<tr>
<td>Cyclophosphamide, cytoxan arabinoside, TBI</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Donor-recipient matching, N (%)</td>
<td></td>
</tr>
<tr>
<td>For sex</td>
<td></td>
</tr>
<tr>
<td>Male/male</td>
<td>10 (37)</td>
</tr>
<tr>
<td>Female/female</td>
<td>5 (19)</td>
</tr>
<tr>
<td>Parous female/male</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Parous female/female</td>
<td>7 (26)</td>
</tr>
<tr>
<td>Nonparous female/female</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplasia; TBI, total body irradiation.

* Parity status of one female donor to male patient is unknown.
This patient did not develop chronic GVHD. FK506 was discontinued in a third patient due to the development of thrombotic thrombocytopenic purpura (TTP) on day 42 post-transplant. This patient received methylprednisolone 1 mg/kg/d as GVHD prophylaxis and did not develop GVHD.

The median peak total bilirubin was 1.5 (0.5 to 10.7) mg/dL. There were 10 patients whose maximum bilirubin was greater than 2.0 mg/dL. Nonfatal hepatic veno-occlusive disease felt to be related to the preparative regimen was diagnosed in one patient. Eleven patients (41%) required antihypertensive agents during the 6-month study period. Eleven patients (41%) required treatment for hyperglycemia. Three of these patients remained insulin-dependent at the end of the 6-month study period. Two patients developed seizures that occurred on day 19 and day 80 posttransplant. FK506 blood levels were 7.9 and 4.6 ng/mL, respectively, and the seizures were felt not to be related to the FK506. Other reported adverse events following transplantation in the trial included diarrhea (96%), fever (89%), nausea (85%), vomiting (81%), and headache (81%).

Treatment response and engraftment. All patients achieved complete remission following allogeneic BMT therapy. All patients had marrow engraftment. The median time to neutrophil recovery (neutrophil count > 500 µL) was 17 (11 to 33) days. Patients were considered platelet and red cell transfusion independent if they did not require transfusions for at least 30 consecutive days. All patients, except one who died of fungal pneumonia at day 53 post-transplant, achieved platelet transfusion independence at a median of 19 (11 to 81) days posttransplant and red cell transfusion independence at a median of 40 (21 to 66) days posttransplant. No patients received hematopoietic growth factors posttransplant.

Acute GVHD. Eleven of the 27 patients developed grade II to IV acute GVHD with a cumulative incidence of 41% (95% CI = 22% to 59%) (Fig 1). One patient (4%) had grade III and none had grade IV acute GVHD. The median time to onset of grade II to IV acute GVHD was 16 (11 to 43) days. Organ involvement associated with grade II to IV acute GVHD included three patients with only skin involve-
T lymphocytes, and the formation of other soluble mediators of the skin, gut, and liver. Activated T cells release interleukin (IL)-2 and interferon-gamma, which activate macrophages to produce inflammatory proteins like tumor necrosis factor-alpha and IL-1, resulting in nonspecific tissue injury leading to direct and indirect cytotoxicity of epithelial cells.

GVHD involves antigen activated T cells and monocytes that are responsible, in part, for the development of acute GVHD.36-40 FK506 was reasonably well tolerated in this study. Renal dysfunction was the most consistent adverse event associated with FK506, resulting in dose reduction. Renal function improved with dose reductions or a temporary hold in the administration of FK506. Renal dysfunction in the postmarrow transplant setting often is exacerbated by the concomitant use of other nephrotoxic drugs frequently used in the management of marrow transplant recipients; thus, monitoring serum creatinine and making appropriate dose adjustments while maintaining adequate blood levels are essential. The spectrum of renal dysfunction associated with FK506 in this trial was similar to CSA monotherapy trials. The mean peak serum creatinine in two CSA monotherapy trials was 2.4 mg/dL, percent of patients with doubling of their serum creatinine from baseline ranged from 58% to 92%, and the percent of patients requiring hemodialysis ranged from 6% (2 of 36 patients) to 16% (8 of 50 patients).1,2 Whether there is a difference in renal toxicity between FK506 and CSA-based immunosuppressive regimes following transplantation can only be determined by a prospective, randomized, controlled trial.

Hepatotoxicity, as evidenced by a median peak total bilirubin of 1.5 mg/dL, was lower than the 2.6 mg/dL reported in a CSA monotherapy trial.1 The incidence of hypertension was similar to reported rates of 39% and 60% of patients in CSA monotherapy trials.1,2 Eleven patients in this trial required antihyperglycemic therapy, three of whom remained insulin-dependent at 6 months posttransplant. The incidence of hyperglycemia with CSA monotherapy is not well documented for comparison. Headaches were commonly reported in our patients, but none were considered severe. However, the dose of FK506 was reduced in one patient with subsequent improvement. Nausea, vomiting, and diarrhea noted in our patients may be secondary to FK506, the pretransplant cytotoxic regimen, or GVHD. Fever is a common consequence of neutropenia after BMT and was expected in this patient population. The 48-year old female patient with myelodysplasia who developed TTP posttransplant received high-dose busulfan and cyclophosphamide before BMT. This patient had full recovery from TTP with plasma-exchange transfusion therapy. TTP or TTP-like syndromes have been associated with FK506 and CSA following solid organ transplantation and with cyclosporine following allogeneic BMT.41-45 The incidence in each clinical setting is uncommon, and the precise pathophysiology is obscure.

FK506 monotherapy was effective in the prevention of GVHD. The overall incidence of acute GVHD in this trial was comparable to that reported in previous studies of CSA monotherapy.1,9 and the incidence of grade III to IV acute GVHD was less.1,2 One patient (9%) required second-line therapy for acute GVHD in this trial, compared with 31%46 and 45%47 in CSA monotherapy studies. The rate of chronic GVHD was relatively low compared with reported rates with cyclosporine monotherapy.1,9 However, the follow-up period in this noncomparative study was limited to 210 days posttransplant.

Pharmacokinetic-parameter estimates are heterogeneous.
as reflected in the large standard deviations. However, the overall results suggest a larger volume of distribution and higher oral bioavailability and clearance than reported in liver transplant patients. Therefore, dosing regimens must be specific to the BMT population and cannot be generalized from other patient groups.

We conclude that FK506 monotherapy has an acceptable safety profile and is effective in the prevention of acute GVHD following HLA-identical sibling marrow transplantation for leukemia and myelodysplasia. No deleterious effects on marrow recovery following transplantation or early (6 months) posttransplant relapse and survival have been observed and follow-up continues. In preclinical studies using a canine model with major histocompatibility complex mismatched donors, methotrexate added to FK506 reduced the incidence of GVHD and improved survival. In Phase II trials with patients receiving marrow transplantation from unrelated donors, FK506 combined with methotrexate or corticosteroids was effective in the prevention of GVHD. Whether FK506 in combination with methotrexate will result in an improvement in the prevention of GVHD compared with cyclosporine in combination with methotrexate following allogeneic sibling-matched marrow transplantation is the subject of an ongoing phase III study. The initial intravenous dose in these subsequent trials has been reduced from 0.04 mg/kg/d to 0.03 mg/kg/d to improve tolerability, particularly dose-limiting nephrotoxicity.

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FK506 (Tacrolimus) monotherapy for prevention of graft-versus-host disease after histocompatible sibling allogenic bone marrow transplantation

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