Marked Increase in Veno-Occlusive Disease of the Liver Associated With Methotrexate Use for Graft-Versus-Host Disease Prophylaxis in Patients Receiving Busulfan/Cyclophosphamide

By James H. Essell, James M. Thompson, Glenn S. Harman, Ronald D. Halvorson, Michael J. Snyder, Robert A. Johnson, and James R. Rubinsak

The use of cyclosporine-A/methotrexate (CyA/MTX) for graft-versus-host disease (GVHD) prophylaxis is safe and effective for patients undergoing allogeneic bone marrow transplantation after preparation with cyclophosphamide and total body irradiation. We report 87 patients prepared for allogeneic transplant with busulfan 4 mg/kg/d orally for 4 days, followed by cyclophosphamide 60 mg/kg/d intravenously for 2 days (Bu4Cy2). A marked increase in hepatotoxicity was observed in 20 patients administered CyA/MTX, compared with 67 historical control patients who received CyA/methylprednisolone (CyA/MP) for GVHD prophylaxis with all other treatment and support variables remaining constant. The incidence of hyperbilirubinemia (bilirubin ≥2 mg/dL) increased from 48% to 80% (P = .02), and the mean maximal bilirubin increased from 4.67 ± 7.27 to 8.72 ± 8.73 mg/dL (P = .04), when CyA/MTX was used in place of CyA/MP for GVHD prophylaxis. In addition, the incidence of veno-occlusive disease (VOD) increased from 18% to 70% (P = .0001), and death caused by VOD increased from 4.5% to 25% (P = .02). Survival was not significantly different for the two groups because of a higher non-VOD death rate in patients receiving CyA/MP for GVHD prophylaxis (P = .77). We suggest caution when using Bu4Cy2 in combination with CyA/MTX for GVHD prophylaxis.

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MATERIALS AND METHODS

All patients were entered on a study protocol that had been reviewed and approved by the Wilford Hall US Air Force (USAF) Medical Center Institutional Review Board.

Preparative regimen. Between October 31, 1986 and June 26, 1990, 90 consecutive patients underwent allogeneic BMT preparation with Bu4Cy2 at Wilford Hall USAF Medical Center. Patients received busulfan orally at a dose of 1 mg/kg of ideal body weight every 6 hours daily for 4 consecutive days (days −7 through −4) for a total dose of 16 mg/kg. Thereafter, CTX was administered intravenously over 1 hour at a dose of 60 mg/kg ideal body weight for 2 consecutive days (days −3 and −2). Donor bone marrow was infused 2 days after the last dose of CTX (day 0). Three patients were not included for further analysis because of the presence of active liver disease, as defined by a bilirubin greater than 2 mg/dL, at the time of transplantation.

Supportive therapy. Before beginning the preparative regimen, all patients were sterilely bathed and placed into strict isolation in laminar air flow (LAF) rooms. Patients remained in LAF until all criteria were met for discharge for outpatient follow-up, or development of medical problems requiring intensive care. Nonabsorbable antibiotics for ‘gut decontamination’ were not used. A ‘low bacterial’ diet was used while in LAF. All patients received commercial Ig preparations at a dose of 500 mg/kg IV beginning on day −8 every 2 weeks until day 100, and orally beginning on day 0 at a dose of 50 mg/kg daily in four divided doses until discharge from LAF. Colony-stimulating factors were not used. All patients received oral clotrimazole and trimethoprim-sulfamethoxazole (T/S). Patients receiving MP for GVHD prophylaxis took one double-strength T/S tablet twice a day beginning on day −7 and continued until their absolute neutrophil count (ANC) was greater than 1000/μL for 2 weeks.

In recent years a number of alternative regimens for transplantation, and GVHD prophylaxis, have been investigated. One such regimen uses busulfan, 4 mg/kg/d orally for 4 days, followed by CTX, 60 mg/kg/d intravenously (IV) for 2 days (Bu4Cy2), using CyA and methylprednisolone (MP) for GVHD prophylaxis.8 VOD has been reported to occur in 2% to 11% of patients so treated.8,9 We now report our experience using Bu4Cy2 showing a marked increase in the incidence, and severity of VOD for patients who received CyA/MTX for GVHD prophylaxis versus CyA/MP.
than 1,000/mm³, after which the schedule was changed to 3 days per week for 1 year, as tolerated. Patients receiving MTX for GVHD prophylaxis received the same dose from days −9 to −2, at which point the drug was stopped until their ANC was greater than 1,000/mm³, with the subsequent schedule the same as above. All blood products transfused were obtained from volunteer donors who had a negative antibody titer against cytomegalovirus. A single intrathecal dose of 12 mg MTX was administered to patients undergoing transplantation for acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML)-lymphoid blast crisis, and acute myelogenous leukemia—French-American-British (AML-FAB) M4/M5 or initial white blood count (WBC) greater than 100,000/mm³.

**GVHD prophylaxis.** Acute GVHD was assessed according to the grading system described in the literature. All patients surviving at least 30 days, or who developed acute GVHD regardless of time of onset, were included in the analysis for GVHD.

Sixty-seven patients who underwent transplant operations between October 31, 1986 and November 22, 1989 received CyA/MP to prevent GVHD. CyA started at midnight of the day of transplantation and was administered as a continuous infusion at the dose of 5 mg/kg/d for 96 hours. Thereafter, the dose was reduced to 3 mg/kg/d administered IV over 6 hours until day 14. Between days 14 and 35, the CyA dose was increased to 3.75 mg/kg administered IV, or orally at four times the IV dose upon discharge. During the initial 35 days, doses were adjusted to maintain the whole blood CyA level between 400 and 1,000 ng/mL. Thereafter, CyA was slowly tapered until day 180, when it was discontinued. MP was started on day 7 at a dose of 0.5 mg/kg/d administered IV. On day 14, the MP dose was increased to 1 mg/kg/d through day 28 and administered orally in an equivalent dose of prednisone on discharge from LAF. Between days 28 and 72, the corticosteroid was slowly tapered and discontinued.

Twenty patients received CyA/MTX as an alternate standard of care for GVHD prophylaxis. Four patients with CML who had transplants between June 8, 1989 and November 22, 1989 were the first to receive CyA/MTX because of an unexplained increased incidence of acute GVHD that we have observed with CyA/MP prophylaxis for patients with CML. After November 22, 1989, the next 16 sequential patients transplanted were administered CyA/MTX in an attempt to reduce the incidence of systemic fungal infections, thought to be related in part to the prolonged use of systemic corticosteroids for GVHD prophylaxis. The first 10 patients received MTX 15 mg/m² IV on day 1, and 10 mg/m² IV on days 3, 6, and 11, with MTX doses held if the serum bilirubin was greater than 2.0 mg/dL, or if the serum aspartate aminotransferase (AST) was greater than two times normal. The next 10 patients received MTX 10 mg/m² on days 1, 3, and 6. Doses of MTX in this group were also held if the bilirubin exceeded two times the bilirubin level obtained on day 0. No MTX was administered if the serum creatinine was greater than 2.0 mg/dL.

**Data collection.** The syndrome of VOD was diagnosed when at least two of the following parameters were present within 30 days after transplant: jaundice (bilirubin > 3.0 mg/dL), hepatomegaly, right upper quadrant pain, and fluid accumulation evidenced by ascites or unexplained weight gain. These parameters were collected prospectively using a computerized database. Clinical computer database entries were merged with the laboratory database to help determine the incidence of VOD.

**Statistical analysis.** Estimates of survival probabilities were computed by using the methods of Kaplan and Meier. Other comparisons used Pearson chi square with Yate’s correction for continuity and Student’s t-tests.
Table 2. Posttransplantation Results

<table>
<thead>
<tr>
<th></th>
<th>CyA/MP</th>
<th>CyA/MTX</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin &gt;2 mg/dL</td>
<td>32/67 (47.8%)</td>
<td>16/20 (80%)</td>
<td>.02</td>
</tr>
<tr>
<td>Day of maximum bilirubin</td>
<td>15.6 ± 7.2</td>
<td>15.7 ± 6.3</td>
<td>.96</td>
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<tr>
<td>(mean)</td>
<td></td>
<td></td>
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<tr>
<td>Mean maximum bilirubin</td>
<td>4.67 ± 7.77</td>
<td>8.72 ± 8.73</td>
<td>.04</td>
</tr>
<tr>
<td>(mg/dL), all patients</td>
<td>(n = 67)</td>
<td>(n = 20)</td>
<td></td>
</tr>
<tr>
<td>Patients with previous</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>intensive chemotherapy,</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ie, AML, ALL</td>
<td>5.19 ± 6.89</td>
<td>11.1 ± 9.38</td>
<td>.04</td>
</tr>
<tr>
<td>(mean)</td>
<td>(n = 45)</td>
<td>(n = 8)</td>
<td></td>
</tr>
<tr>
<td>Other, ie, CML, MDS*</td>
<td>2.90 ± 4.64</td>
<td>7.16 ± 7.48</td>
<td>.05</td>
</tr>
<tr>
<td>(mean)</td>
<td>(n = 22)</td>
<td>(n = 12)</td>
<td></td>
</tr>
<tr>
<td>Incidence of VOD</td>
<td>12/67 (17.9%)</td>
<td>14/20 (70%)</td>
<td>.0001</td>
</tr>
<tr>
<td>Previous intensive</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>chemotherapy*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death secondary to VOD,</td>
<td>3/67 (4.5%)</td>
<td>5/20 (25%)</td>
<td>.02</td>
</tr>
<tr>
<td>all patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence of systemic</td>
<td>14/67 (20.1%)</td>
<td>2/20 (10%)</td>
<td>.44</td>
</tr>
<tr>
<td>fungal infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death secondary to fungi</td>
<td>13/67 (19.4%)</td>
<td>2/20 (10%)</td>
<td>.52</td>
</tr>
<tr>
<td>Incidence of GVHD ≥ grade</td>
<td>20/61 (32.8%)</td>
<td>2/11 (18.2%)</td>
<td>.54</td>
</tr>
<tr>
<td>III/IV</td>
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<td></td>
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</tbody>
</table>

*Differences between patients with prior chemotherapy, compared with those without prior chemotherapy, using the same GVHD prophylaxis regimen, were not significant.

 Patients were eligible for evaluation if their donor was less than a two antigen mismatch, if they survived more than 30 days posttransplant and had evidence of engraftment, or if they had acute GVHD.

Fig 1. Mean daily bilirubin posttransplant for 67 patients administered CyA/MP compared with 20 patients administered CyA/MTX for GVHD prophylaxis.

Fig 2. Actuarial survival for 67 patients administered CyA/MP (---) compared with 20 patients administered CyA/MTX (-----) for GVHD prophylaxis.

DISCUSSION

VOD of the liver complicating BMT was first described in 1979. VOD occurs after transplant preparation with many different cytotoxic drug and/or TBI containing regimens. The incidence of VOD was 21% in 255 patients transplanted for hematologic malignancies using a variety of preparative regimens. In a series of 235 patients treated with CTX and TBI (Cy/TBI), VOD was the third leading cause of death, after GVHD and infection. Indeed, hepatic dysfunction was the most common regimen-related toxicity reported in a review of patients treated with Cy/TBI, with the observation that all patients who developed grade III-IV liver toxicity died before day 100. The clinical diagnosis of VOD has been shown to be predictive of histologic evidence of VOD in approximately 90% of cases. VOD occurs after transplant preparation with many different cytotoxic drug and/or TBI containing regimens. The incidence of VOD was 21% in 255 patients transplanted for hematologic malignancies using a variety of preparative regimens. In a series of 235 patients treated with CTX and TBI (Cy/TBI), or BuCy, VOD was the third leading cause of death, after GVHD and infection. Indeed, hepatic dysfunction was the most common regimen-related toxicity reported in a review of patients treated with Cy/TBI, with the observation that all patients who developed grade III-IV liver toxicity died before day 100.
Drugs other than those used in the preparative regimen can also contribute to hepatic toxicity in patients undergoing allogeneic BMT. MTX hepatotoxicity was first described in 1955 manifesting as an increased prevalence of hepatic fibrosis at autopsy among children who had received MTX as part of their treatment for leukemia.9 Since that time, numerous descriptions of hepatic injury caused by MTX have been reported. The hepatic effects have included steatosis, nuclear pleomorphism, sinusoidal dilatation, as well as fibrosis and cirrhosis.20-23

When MTX was added to CyA for acute GVHD prophylaxis in leukemic patients treated with Cy/TBI, a significant increase in bilirubin levels was observed during the first 14 days after marrow infusion in patients who received CyA/MTX compared with CyA alone. The mean peak bilirubin observed increased from 2.6 mg/dL, in patients receiving CyA alone, to 3.9 mg/dL in patients treated with CyA/MTX (P = .015).11,24,25 Importantly, this elevation of bilirubin was not associated with an increased incidence of VOD, mortality, or length of hospital stay. When the CyA/MTX combination was used for GVHD prophylaxis for patients with severe aplastic anemia, a group of patients historically at low risk for hepatotoxicity caused by pretransplant conditioning with CTX alone, an increased incidence of hyperbilirubinemia has been observed posttransplant.26,27 Of note, one patient who received CyA/MTX died of VOD.

The reported incidence of VOD after transplant preparation with Bu4Cy2 to date is lower than that published for Cy/TBI. Tutschka et al8 reported 50 patients with hematologic malignancies, administered CyA/MP for GVHD prophylaxis, with a VOD rate of only 2%. Using the same regimen, Brodsky et al9 reported a 10.8% incidence of VOD in 37 patients. MTX was not used to prevent GVHD in either series.

Our initial 67 patients, administered the identical Bu4Cy2 preparative regimen and CyA/MP GVHD prophylaxis, experienced a baseline incidence of VOD of 18%. Whereas this was comparable to Cy/TBI treated cohorts, systemic fungal infections occurred in 20% of our patients, of whom 93% subsequently died as a result. In an attempt to decrease fungal morbidity and mortality we decided to investigate the role of CyA/MTX for GVHD prophylaxis, reasoning that prolonged corticosteroid administration according to the CyA/MP GVHD prophylactic regimen was the most likely risk factor for the development of fungal infection. The change to CyA/MTX for GVHD prophylaxis did result in a nonsignificant decrease in the incidence of systemic fungal infection and deaths caused by fungemia. However, a marked concomitant increase in the incidence of VOD was observed without any change in other supportive measures. The first 10 patients of the CyA/MTX cohort received the traditional IV MTX dosing.11 As seven of these patients developed VOD, we decreased the dose of MTX for the next 10 patients per Deeg et al.12 No significant decrease in VOD occurred, as seven of these next 10 patients receiving this MTX dose schedule also developed VOD. Although a statistically significant difference in the incidence of death from VOD was noted in patients receiving CyA/MTX (25%) versus those receiving CyA/MP (4.5%) (P = .02), overall survival was not significantly different between the two groups (Fig 2). Although not statistically significant, this observation may in part be attributable to the lower incidence of acute GVHD (18.2% v 32.8%) (P = .54), and systemic fungal infection (10% v 20%) (P = .43) for those receiving CyA/MTX versus CyA/MP, respectively.

Several factors should be explored in an attempt to explain the marked increase in the incidence of VOD for our patients who received CyA/MTX for GVHD prophylaxis. First, there is the possible VOD ‘preventative’ role that steroids may have when begun on day 7 posttransplant that, when omitted, increase the risk for VOD. We have treated 11 patients with the identical Bu4Cy2 regimen in preparation for autologous BMT for acute myelogenous leukemia. A bilirubin greater than 2.0 mg/dL was noted in 3 of 11 (27.3%); 2 of 11 (18.2%) developed clinical VOD, and the mean maximum bilirubin for the group was 3.56 ± 4.69 mg/dL. These results are very similar to our allogeneic experience using Bu4Cy2 with CyA/MP GVHD prophylaxis, suggesting that the omission of corticosteroids had no effect on the incidence of hyperbilirubinemia or VOD.

A second possibility is that the combination of CyA/MTX is more hepatotoxic when the CyA is dosed according to the CyA/MP schedule, ie, 5 mg/kg for the first 96 hours posttransplant, which amounts to a 40% increase in dose intensity. CyA is known to be hepatotoxic.28 Although we realize the potential problems of comparing cohorts from other series, the Vancouver group has treated 57 patients with the identical Bu4Cy2 regimen using ‘standard’ doses of CyA/MTX noting a 51% incidence of grade II-IV hepatotoxicity.18,29

The last factor that must be considered is the potential for differential oral absorption of busulfan, which can produce a marked variation in pharmacokinetics between patients as reported by Grochow et al.30 This study showed that the risk for VOD may be related to total drug exposure assessed by ‘area under the curve’ calculation. Future studies will have to properly address this when evaluating causal factors for VOD in patients receiving busulfan as a part of the preparative regimen.

CyA/MTX is an effective regimen for acute GVHD prophylaxis and has been shown to have acceptable toxicity when used following the Cy/TBI preparative regimen. While patients receiving CyA/MP and CyA/MTX for GVHD prophylaxis with the Bu4Cy2 preparative regimen had similar overall survival in our experience, hepatotoxicity was potentially severe with the use of CyA/MTX, and resulted in a significant increase in death resulting from VOD. Physicians using Bu4Cy2, or other busulfan containing preparative regimens must be aware of the potential for serious liver toxicity if CyA/MTX is used for GVHD prophylaxis. Additionally, investigators designing dose escalation protocols containing busulfan may wish to consider using GVHD prophylactic regimens that do not contain MTX. Future properly designed prospective randomized trials are warranted to study these issues.
REFERENCES

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