The Effect of Mycophenolate Mofetil on Graft Versus Host Disease in Cyclosporine Induced Thrombotic Microangiopathy after Allogeneic Hematopoietic Stem Cell Transplantation

Niparuck P[1*], Pukiat S[1], Puavilai T[1], Ativitavas T[2] and Ungkanont A[1]

[1]Division of Hematology, Medicine department, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[2]Division of Oncology, Medicine department, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

*Corresponding author: Pimjai Niparuck, Clinical Instructor, Division of Hematology, Medicine department, Ramathibodi Hospital, Mahidol University, Rama VI Road, Bangkok 10400, Thailand, Tel: +662-201-1392, +662-201-2373; Fax: +662-201-1392; E-mail: niparuckblue@gmail.com

Abstract

Calcineurin inhibitor induced thrombotic microangiopathy (TMA) is a serious complication in hematopoietic stem cell transplantation (HSCT). Mycophenolate mofetil (MMF) is a safe immunosuppressive drug that can be used for prophylaxis of primary graft versus host disease (GVHD) after developing TMA. In this study, our two patients received CellCept® after developing cyclosporine induced TMA. Acute GVHD (skin, oral and liver) was found in both patients, nevertheless, the GVHD was not severe and can be controlled with low dose prednisolone. Therefore, MMF is an optional immunosuppressant for GVHD prophylaxis in the transplant patient diagnosed with calcineurin or m-TOR inhibitors induced TMA.

Keywords: Thrombotic microangiopathy; Mycophenolate mofetil; Graft versus host disease

Background

Thrombotic microangiopathy (TMA) is a serious complication following hematopoietic stem cell transplantation (HSCT). It can occur after graft versus host disease (GVHD) as an opportunistic infection, calcineurin inhibitors (CNIs) or mammalian target of rapamycin (m-TOR) inhibitor therapy for GVHD prophylaxis [1-4]. Tacrolimus (FK-506) is a calcineurin inhibitor and is more potent than cyclosporine and binds to a different immunophilin (FK-binding protein) to inhibit calcineurin resulting in decreased production of interleukin-2 and reduction of T cell proliferation. Whereas CellCept®

(mycophenolate mofetil) is the 2-morpholinoethyl ester of mycophenolic acid (MPA). Three different diagnostic criteria for TMA approved by European Leukemia Net (ELN), Bone and Marrow Transplant Clinical Trials Network (BMT CTN) and overall thrombotic microangiopathy (O-TMA) grouping have been proposed which are based on the number of schistocytes and platelets, levels of lactate dehydrogenase (LDH), haptoglobin and creatinine, normal coagulogram, negative direct Coombs test (DCT) and required transfusion [5-7]. Currently there is no definite recommendation for primary GVHD prophylaxis in patient diagnosed with cyclosporine induced TMA after HSCT. Therefore we report our two patients who received treatment with mycophenolate mofetil (MMF) as primary GVHD prophylaxis following cyclosporine induced TMA after HSCT.

Patient	Sex	Age (y)	Cytogenetic	Prior therapy (No. of cycles)	Donor/(No. of HLA match)	Disease status at HSCT	Conditioning Regimen	GVHD prophylaxis
1	F	28	46, XX	7+3 (2)	Father (9/10)	BM blast (5-10%)	IV Flu/Bu/ATG	MTX/CSA
2	M	35	T (3;3)	7+3 (1) HiDAC (4) MEC (1)	Sister (10/10)	CR2	IV Flu/Bu	MTX/CSA
HiDAC: High-dose Cytarabine; Flu/Bu/ATG: Fludarabine/Busulfan/Alemtuzumab; Flu/Bu: Fludarabine/Busulfan; MTX/CSA: Methotrexate/Cyclosporine								

Table 1: Patient characteristics and treatment regimen.

Mycophenolic acid (MPA) is an active form of MMF, which can inhibit T and B lymphocyte proliferation by inhibition of inosine-5'-monophosphate dehydrogenase enzyme inducing guanine nucleotide synthesis. MMF is used worldwide in organ transplantation in combination with cyclosporine and corticosteroids. This triple immunosuppressive therapy has shown promising outcomes in

marked reduction of acute rejection during the first six months after transplantation. The target area under the plasma concentration-time curve (AUC) for the early post-transplantation period in patients treated with MMF and cyclosporine and the target AUC for GVHD prophylaxis with tacrolimus+CellCept® in HSCT patients are about 30-60 µgh/ml [8]. Nash et al. also demonstrated that MMF in

combination with cyclosporine was an effective primary GVHD prophylaxis regimen in HSCT, the median MPA level were in range of 2.8-3.1 μg/ml which were consistent with therapeutic window described from the solid organ transplantation studies [9-11]. Moreover, the trough level of MPA>0.5 μg/ml is significantly effective for treating acute GVHD in HSCT patients receiving CellCept® and corticosteroids combination therapy [12].

Both patients were diagnosed with acute myeloid leukemia. They were treated with chemotherapy and underwent allogeneic HSCT, the patients' characteristics and treatment regimens are shown in Table 1. Both patients developed cyclosporine induced TMA which occurred 22 and 28 days after donor stem cell infusion, respectively. In this study, TMA was defined by the O-TMA criteria which included schistocytes >2 per high-power field, platelet count <50 × 10⁹/l or <50% of normal baseline, increased LDH level, low haptoglobin level, negative DCT and normal coagulogram [7].

Case Presentation

Patient 1

Patient 1 was diagnosed with TMA on day 22, she had no platelet engraftment (platelet count <20 × 10⁹/l) and required periodic platelet transfusions, whereas neutrophil engraftment (ANC >0.5 × 10⁹/l) was on day 12. The complete blood count (CBC) at time of diagnosis of TMA revealed anemia (hemoglobin; Hb 9 g/dl), thrombocytopenia (platelet count 12 × 10⁹/l) and leukocytosis (white blood cell; WBC 15 × 10⁹/l). Reticulocyte count was increased (7%), LDH level was high, 323 IU/L (normal, 100-190 IU/L) while haptoglobin level was normal, 1.2 mg/ml (normal, 0.3-2.1 mg/ml). Microangiopathic hemolytic anemia (MAHA) was found on peripheral blood smear. ADAMTS13 level was 71%. The DCT was negative and there was no prolonged APTT, PT (INR) or TT. Serum creatinine, indirect and total bilirubin levels were normal, she had no fever or neurological disorder. The platelets and Hb level were greater than 100 × 10⁹/l and 10 g/dl within 9 and 7 days after cyclosporine discontinuation, respectively.

Oral CellCept® 1,500 mg twice daily (60 mg/kg) were initiated for prevention of GVHD after diagnosis of TMA, aim to maintain trough MPA level of 3-4 μg/ml. The trough MPA level was 4.8 μg/ml after 5 days of CellCept® 3,000 mg/day. The dose was decreased to 2,000 mg/day. The serial trough MPA level was in range of 3.2-3.8 μg/ml. After 4 weeks of CellCept® therapy (d+50), the patient developed polyomavirus BK (BK virus) hemorrhagic cystitis while taking CellCept® 1,500 mg/day and MPA level was 4.3 μg/ml. She was treated with 100 mg/day of leflunomide (Arava) for 5 days and then decreased dosage to 20 mg/day, ciprofloxacin 1,000 mg/day and CellCept® decreased to 1,250 mg/day. Unfortunately, she developed stage I oral and also stage I liver acute GVHD one month later (d+81), MPA level was 3.9 μg/ml. Low dose prednisolone (20 mg/day) was started to control GVHD, the clinical of oral and liver GVHD had improved even reducing dose of prednisolone. However, BK viremia was occurred, BK viral load in peripheral blood (PB) was 156,569 copies/ml and she also got worsening hematuria at 5 months after HSCT while she was taking CellCept® 1,250 mg/day and prednisolone 5 mg/day. The clinical presentation of hematuria and BK viremia had improved after adding treatment with intravenous (IV) cidofovir 1 mg/kg weekly for 2 weeks. Finally the clinical presentation of GVHD and BK infection has gradually improved and currently (19 months after HSCT), she has taken only CellCept® 500 mg/day and prednisolone 5 mg/day, her latest MPA level was 1.5 μg/ml, BK viral load in both the PB and midstream urine (MSU) were negative, viral load <10 copies/ml. Leflunomide and ciprofloxacin were discontinued already for 7 months.

Patient 2

Patient 2 was diagnosed with TMA on day 28; he achieved neutrophil and platelet engraftment on day 15. The CBC (day+24) revealed mild anemia (Hb 11.5 g/dl), WBC and platelet counts were 9.8 and 187 × 10⁹/l, respectively, nevertheless, he developed thrombocytopenia (platelet count 90 × 10⁹/l) after 28 days of donor stem cell infusion and then developed petechial hemorrhage on all his extremities and platelet count went down to 9 × 10⁹/l at 32 days after HSCT. The CBC at time of diagnosis of TMA revealed anemia (Hb 10.5 g/dl) and WBC count was 3.4 × 10⁹/l. Reticulocyte count was 0.8%, LDH level was high, 266 IU/L (normal, 100-190 IU/L) and haptoglobin level was normal, 0.9 mg/ml (normal, 0.3-2.1 mg/ml). MAHA was found and ADAMTS 13 level was 93%. The DCT was negative and normal coagulogram was seen. Serum creatinine, indirect and total bilirubin levels were normal, he had no fever or neurological disorder. Bone marrow study showed normocellular marrow, adequate megakaryocytes, myeloid and erythroid progenitor cells. There was no evidence of relapsed AML by flow cytometry analysis. The platelets were greater than 20 and 100 × 10⁹/l after discontinuing cyclosporine for 4 and 20 days, respectively.

Oral CellCept® 750 mg twice a day (30 mg/kg) was given after diagnosis of TMA. He developed stage I skin GVHD (maculopapular rash) and liver GVHD (stage I) on day+89, MPA level was 3.2 μg/ml. Prednisolone 30 mg/day was started. The clinical of skin and liver GVHD had significantly improved until day+135, he developed BK viral cystitis during taking CellCept® 1,000 mg/day and prednisolone 15 mg/day, MPA level at that time was 2.8 μg/ml. He was treated with leflunomide 100 mg/day for 5 days followed by leflunomide 20 mg/day. Levofloxacin 500 mg/day, 400 mg/kg of IV immunoglobulin were administered and prednisolone was reduced to 10 mg/day. A single dose of IV cidofovir 1 mg/kg was administered 2 months later due to no improvement in the clinical presentation of cystitis. The clinical presentation of cystitis gradually improved after antiviral therapy, unfortunately, the patient skin GVHD (erythroderma) worsened. The tropical steroids, antibiotic and increasing dosage of prednisolone from 10 to 15 mg were given, the skin GVHD has gradually improved which this improvement had taken over 5 months. Currently (1 year after HSCT), he has no clinical of cystitis and MSU BK viral load was negative, he has taken only CellCept® 750 mg/day and prednisolone 10 mg/day.

Discussion

This study indicated that TMA in both patients responded to withdrawal of cyclosporine without plasma exchange. The result was consistent with the proposed mechanism of CNI induced TMA which appeared to be linked to the endothelial cell toxicity. Cyclosporine and tacrolimus have been shown to induce the synthesis of thromboxane A2 (TXA2) and decrease the endothelial cell prostacyclin (PGI2) production causing platelet aggregation [13-15]. The addition of sirolimus to CNI has been associated with loss of endothelial cell integrity, and formation of a proinflammatory and procoagulant state leading to secondary TMA [16,17]. We treated these patients with CellCept® alone as primary GVHD prophylaxis after developing TMA as the treatment with other immunosuppressants such as tacrolimus or sirolimus (a potent inhibitor of antigen-induced proliferation of T cells, B cells, and antibody production) has been reported of TMA.

Our target MPA level was 3-4 µg/ml following the therapeutic window reported in the previous studies; even they used the combination of MMF and CNIs [10-12]. Nevertheless our patients still developed GVHD after receiving primary GVHD prophylaxis with CellCept® alone, even MPA levels at the time of GVHD diagnosis in both patients (1 and 2) were in therapeutic levels, 3.9 and 3.2 µg/ml respectively. These data proved that single therapy with CellCept® was not enough for prevention of GVHD as described in the previous studies; however, the clinical of GVHD in our patients were not an extensive stage and were manageable with adding low dose prednisolone therapy [18-21]. Viral infection especially BK virus in this study was occurred after treatment with CellCept® either taking CellCept® alone or in combination with steroids and had spent a long time in therapy which our result was in congruence with those of the previous reports [22-23]. In contrast, there was no complication of bacterial, cytomegalovirus or adenoviral infection, gastrointestinal irritation, thrombocytopenia, anemia or leucopenia in our study

Conclusions

CellCept® is an optional immunosuppressive drug for GVHD prophylaxis in HSCT patients who developed TMA after using calcineurin or m-TOR inhibitors. The efficacy of GVHD therapy is increased after using CellCept® in combination with corticosteroids, nevertheless, the complication of viral infection is of concern and need to be monitored closely.

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