Transpl Int (2003) 16: 202–206
DOI 10.1007/s00147-002-0487-9

CASE REPORT

Rituximab in association with rapamycin for post-transplant lymphoproliferative disease treatment

Valter Duro Garcia
José Luz Bonamigo Filho
Jorge Neumann
Laura Fogliatto
Anna Maria Geiger
Clotilde Druck Garcia
Vivianne Barros
Elizete Keitel
Antonio Eduardo Bittar
Auri Ferrera des Santos
Sergio Roithmann

Received: 9 October 2001
Revised: 12 July 2002
Accepted: 22 August 2002
Published online: 13 February 2003

V.D. Garcia (✉) · J.L.B. Filho
J. Neumann · L. Fogliatto · A.M. Geiger
C.D. Garcia · V. Barros · E. Keitel
A.E. Bittar · A.F. des Santos
S. Roithmann
Santa Casa Hospital,
Rua Correa Lima 1493,
90850 Porto Alegre, Brazil
E-mail: cdgarcia@terra.com.br
Tel.: +55-51-32280496

Abstract Post-transplant lymphoproliferative disease (PTLD) is an uncommon but life-threatening complication of solid-organ and blood stem-cell transplants. It responds poorly to therapy, including reduction of immunosuppression, interferon, antivirals or chemotherapy. Small series of PTLD successfully treated with rituximab have been reported, and experimental studies suggest that rapamycin inhibits growth of human Epstein-Barr virus-transformed B lymphocytes. We report two cases of PTLD after renal transplantation that were successfully treated with rituximab in association with rapamycin. This report suggests that rituximab associated with rapamycin could be an effective and safe treatment for PTLD.

Keywords Post transplant lymphoproliferative disease · Rituximab · Rapamycin · Transplantation

Introduction

Post transplantation lymphoproliferative disorder (PTLD) is caused by an uncontrolled expansion of B lymphocytes infected with the Epstein-Barr virus (EBV). It is one of the most serious complications occurring after organ transplantation [28, 29]. PTLDs comprise a spectrum of lymphoproliferative disorders ranging from a polyclonal atypical lymphoid hyperplasia to a monoclonal, overtly malignant, B-cell lymphoma [2]. Although relatively uncommon, the risk of developing lymphoma after transplantation has been reported to be 28–49 times higher than in the general population, and it is believed to be responsible for 16% of tumours in transplant patients [10, 11].

The prevalence ranges from 1%–10%. It depends on many factors, including allograft type, Epstein-Barr virus serological status before transplantation, adult vs paediatric population, underlying disease, and degree and duration of immunosuppression. Additional risk factors include lymphoid cell phenotype at transplantation, cytomegalovirus (CMV) infection, and cytokine promoter gene polymorphism [2, 25, 30].

A defect in T-cell regulation allows uncontrolled proliferation of B (90% of cases) or other T lymphocytes in response to a viral infection, particularly EBV (90%–95% of PTLDs are positive for EBV). This occurs as a consequence of prolonged administration of immunosuppressive drugs [2].

PTLD prognosis is generally poor under traditional treatment, including reduction of immunosuppression, administration of antiviral agents or chemotherapy. There are some recent reports of PTLD remission with administration of rituximab, a monoclonal chimeric antibody directed against the B-cell-specific antigen CD20 [1, 5]. However, relapses are frequent, and the withdrawal of immunosuppressive drugs usually leads to graft loss.

Two experimental studies have suggested that rapamycin, a potent immunosuppressive drug, inhibits B-cell lymphoma in vitro [14, 16]. These observations have prompted us to test the drug in this setting, allowing continued immunosuppression with a lower risk of PTLD relapse. To our knowledge, this is the first clinical study exploring this approach. We present here our first two cases of PTLD successfully treated with rituximab followed by rapamycin.

Case reports

Case 1

In November 1998, a 15-year-old youth presented with rapidly progressive glomerulonephritis that was refractory to high-dose corticosteroid treatment. He was started on haemodialysis in December 1998 and underwent cadaveric renal transplantation on 30 September 1999. CMV serology was negative for the recipient and positive for the donor. The prophylactic immunosuppressive regimen was a triple therapy comprising cyclosporine, 8 mg/kg/day, aiming at a blood concentration around 1,200 ng/ml at peak level – 2 h; azathioprine (2 mg/kg/day); and prednisone (20 mg/day up to the 3rd month, tapering 2.5 mg/kg monthly up to the 6th month). Neither monoclonal nor polyclonal antibodies were induced.

The patient presented with delayed graft function requiring haemodialysis for 3 weeks. His first renal biopsy on day 7 showed acute rejection grade I and acute tubular necrosis (ATN) (Banff classification). He received 500 mg/day of methylprednisolone (MP) for 3 days, and azathioprine was switched to mycophenolate mofetil (1.5 g/day). His second renal biopsy on day 15 showed acute rejection grade II and ATN. He received 500 mg/day of MP for 2 days, and cyclosporine was switched to tacrolimus (an initial dose of 0.2 mg/kg/day, aiming at blood levels of 10–15 ng/ml). On day 18 he started diuresis, quitting haemodialysis on day 21. The third renal biopsy on day 24 was considered as borderline. He was discharged on day 28 as asymptomatic, normotensive, and with a serum creatinine level of 4.0 mg/dl. His serum creatinine declined to 2.0 mg/dl. The immunosuppressive regimen was tacrolimus (10 mg/day, (blood levels 7–10 ng/ml), mycophenolate mofetil (1 g/day) and prednisone (10 mg/day).

At the 4th month, a submandibular lymph node was observed, which spontaneously remitted after a week. At the 6th month, new lymph nodes were noted in the same region. Abdominal computed tomography (CT) scan showed mediastinal and celiac lymphadenopathies, multiple hepatic nodes, and intestinal infiltration. The lymph node biopsy showed a diffuse polymorphic CD20+ B-cell lymphoma. Tumour cells did not express surface immunoglobulin, and clonality could not be defined. Bone marrow biopsy and cerebral CT were normal. He had Ann Arbor stage IV disease. The age-adjusted international prognostic index (IPI) was 2 (high-intermediate risk).

Immunosuppressive therapy was reduced; mycophenolate was stopped, the tacrolimus dose decreased from 10 to 6 mg/day, and acyclovir was started at 1,200 mg/d. There was no remission of PTLD in 2 weeks. A renal biopsy was done 1 week after reduction of immunosuppression, showing acute rejection grade II. MP (250 mg/day) was given for 3 days.

He was administered rituximab at 4 weekly doses of 375 mg/m^2. No adverse effects were observed, and complete remission of submandibular lymphadenopathy was achieved in the 1st week. In 3 months, the hepatic and intestinal lesions disappeared. Tacrolimus was stopped, and rapamycin was started at 2 mg/day, aiming to avoid relapse of PTLD and to maintain graft function. At present, 24 months after PTLD treatment, he is asymptomatic without any sign of PTLD, with a serum creatinine level ranging from 1.5–1.7 mg/dl. His immunosuppressive regimen comprises prednisone (10 mg/day) and rapamycin (3 mg/day, blood level: 10–15 ng/ml).

Case 2

A 17-year-old Caucasian youth with end-stage renal disease secondary to obstructive uropathy (posterior urethral valve), received a renal transplant from a cadaveric donor in January 2001. His immunosuppressive protocol included basiliximab (20 mg on 0 and 4th day), tacrolimus (0.2 mg/kg/day), prednisone (20 mg/day) and azathioprine (2 mg/kg/day). He had no acute rejection episodes.

The patient was discharged 15 days after the procedure without any complications and with a serum creatinine level of 1.5 mg/dl. He showed an increase in serum creatinine 1 month later and was submitted to graft biopsy, which showed nephrotoxicity. The tacrolimus dose was decreased (the highest level was 18 ng/dl) and serum creatinine went down to 1.5 mg/dl.

In June 2001, the patient was admitted with fever, abdominal pain, leukopenia, and a serum creatinine level of 2.5 mg/dl. He was pale, without palpable lymph nodes and with a normal chest film. His CMV antigenaemia was positive, and renal biopsy was normal. A gastro-duodenal endoscopy showed a tumour in the duodenum. Pathological and immunohistochemical studies were consistent with a diffuse polymorphic CD20+ B-cell non-Hodgkin lymphoma, heavily infiltrated with T cells. Surface immunoglobulin light-chain expression was negative. Abdominal CT scan showed an extensive tumour mass in the coeliac region. Thoracic CT scan was normal. Bone marrow biopsies were negative. Clinical staging was IIe (Ann Arbor). Age-adjusted IPI was 2 (high–intermediate risk category).

The CMV infection was treated with ganciclovir. Immunosuppressive therapy was initially reduced (azathioprine stopped, and the tacrolimus dose reduced to 3 mg/day), then replaced by treatment with rapamycin at 2 mg/day (level 10 ng/ml). CMV antigenaemia became negative within 10 days of administration of ganciclovir. After 2 weeks, the abdominal pain disappeared, but he still suffered from diarrhoea and weight loss. A new endoscopy showed tumour growth.

The patient was treated with 4 weekly doses of 375 mg/m^2 of rituximab. He had no severe adverse effects, except for a hypersensitivity reaction during the first infusion, including rash, cough, and wheezing. One month after completion of anti-CD20 therapy, only a minimal residual tumour was detected endoscopically. It was negative for malignant cells when biopsied. Abdominal ultrasonic examination (US) showed complete regression of the mass previously noted. After 12 months of follow-up, the patient is asymptomatic, with a serum creatinine level of 1.5 mg/dl, and a normal abdominal CT scan.

Discussion

In this paper, we describe two patients with PTLD successfully treated with rituximab and rapamycin. Rituximab was used to induce clinical remission, and rapamycin was used in an attempt to prevent graft rejection and also PTLD relapse.

Treatment of PTLD is a difficult issue. Various approaches have been attempted, but most of the published series are small, and histological categorisation is inconsistent. Withdrawal or reduction of iatrogenic immunosuppression can produce clinical remissions in up to 50% of patients [20, 29], but this approach is rarely successful in patients with widespread PTLD, whether polyclonal or monoclonal. Moreover, there is considerable risk of rejection, and eventually, graft loss. Anecdotal reports suggest that tumours regress with anti-EBV therapy with high-dose acyclovir [9]. However, treatment is generally ineffective, because EBV is not replicative in infected B cells. Recently, pharmacological induction of latent viral thymidine kinase (TK) gene and enzyme in tumour cells using arginine butyrate, followed

by treatment with ganciclovir, has produced clinical responses in previously refractory patients [4]. Interferon-α has been effective in some cases, but might increase the risk of rejection [20, 24].

Results of antiblastic chemotherapy in PTLD have, in general, been disappointing. Although some centres have reported high response rates, most have shown poor results with high morbidity and mortality [2]. Among marrow transplant recipients, two anti-B-cell antibodies (anti-CD21 and anti-CD24) induced a near 50% complete response rate with long-term survival in some patients with oligoclonal disease [6].

More recently, anti-CD20 monoclonal antibody therapy (rituximab) became available and showed promising clinical activity in different types of malignant lymphoproliferation. A high response rate of rituximab in PTLD has been reported by different groups (Table 1), generally without significant toxicity.

Rituximab is a unique monoclonal antibody for the treatment of non-Hodgkin's lymphoma. This chimeric mouse/human antibody was discovered in 1991. It is a human IgG1 kappa antibody with mouse variable regions isolated from a murine anti-CD20 antibody, IDEC-2B8, that binds with high affinity to cells expressing the CD20 antigen found on the surface of malignant and normal B cells, but not on other normal tissues. It mediates complement-dependent cell lyses in the presence of human complement, and antibody-dependent cellular cytotoxicity with human effector cells [21]. The Food and Drug Administration (FDA) approved rituximab in 1997 and the European Union (EU) in 1998 for the treatment of relapsed or refractory, CD20-positive, B-cell, low-grade or follicular non-Hodgkin's lymphoma. It is the first therapeutic monoclonal antibody approved for the treatment of cancer and the first single agent approved specifically for lymphoma therapy [7].

Our case reports further confirm the activity of rituximab in PTLD. Insignificant toxicity makes rituximab a very attractive alternative in managing the condition of these particular patients. Most adverse effects associated with rituximab are mild to moderate. Infusion-related reactions occur more commonly during initial infusion. Haematological effects are generally mild and transient, and adverse immune responses are rare [7, 12].

Maintenance of immunosuppressive therapy in PTLD patients is a difficult issue. Some degree of immunosuppression is necessary to preserve graft function, but on the other hand, relapses of PTLD might be associated with the absence of efficient cytotoxic T cells.

Rapamycin, a macrolide antifungal antibiotic isolated from *Streptomyces hygroscopicus*, has potent immunosuppressive properties. It blocks the cellular cycle progression from phase G_1 to S, by inhibition of some signal transduction pathways [22]. Besides its inhibitory effects on normal cells of the immune system, rapamycin also inhibits proliferation of transformed cell lines. Various cell lines can be affected, including those of the central nervous system and liver, melanocytes and osteoblasts, and miogenic, renal, and connective-tissue cells, as well as T and B cells transformed by human T-lymphotropic virus 1 (HTLV-I) and EBV, respectively [3].

Experimental data suggest that the anti-proliferative effect of rapamycin could be cell-type specific: inhibitory concentration (IC_{50}) was low in lymphoma B-cell lines (BKS-2, L1.2, NFS 1.1, and WEHI-279), thymoma and rabdomyosarcoma [16]. In contrast, the inhibitory effect was much less marked in human melanoma and carcinoma cell lines [16]. Everolimus (RAD) has a high inhibitory effect on in-vitro growth of six different PTLD-like EBV+ lymphoblastoid B-cell lines. Similarly to normal T cells, RAD blocked cell-cycle progression in PTLD-like B cells in the early (G_0/G_1) phase. The drug also had a profound inhibitory effect on the growth of PTLD-like EBV+ B cells xenotransplanted into severe combined immunodeficiency (SCID) mice. In this in-vivo xenotransplant model, RAD markedly delayed growth or induced regression of the established tumours [16].

Table 1 Treatment of PTLD with rituximab (*CR* complete remission)

Author	*n*	Allograft type	PTLD localisation	Result
Faye et al., 1998 [5]	1	Bone marrow	Right tonsil mass	CR
Cook et al.,1999 [1]	3	Lung		CR: 2 Non-response: 1
Grillo-Lopez et al., 1999 [8]	6	Liver (2), kidney (1), lung (1), heart (1)		Remission: 5
Milpied et al., 1999 [15]	3 2			CR: 14/26 (54%) Partial remission: 4/26 (15%)
Kuehnle et al., 2000 [12]	3	Bone marrow	Adenopathy Hepatomegaly Pulmonary infiltrates	CR: 3
Oertel et al., 2000 [19]	1	Liver	Gastric and parasplenic	CR
Niedermeier et al., 2000 [17]	1	Lung	Lung mass	CR
O'Dwyer et al., 2000 [18]	1	Kidney-pancreas	Brain and liver	CR (?)
Skoda-Smith et al., 2001 [27]	1	Blood stem cells		CR

Cell death induced by rapamycin in BKS-2 lymphoma was found to be via apoptosis induction [23, 26]. Tacrolimus, but not cyclosporine, antagonised the effect of rapamycin [16].

In conclusion, these studies suggest that macrolides such as rapamycin or RAD might be useful in the clinical management of PTLD, which prompted us to test them in combination with anti-CD20 therapy. To our knowledge, this is the first report of this clinical approach to PTLD.

Because only two patients with limited follow-up are described, the success of this approach needs further confirmation; moreover, the individual roles of rapamycin and rituximab in PTLD need to be established. Also, molecular studies to detect EBV genoma, analysis of tumour clonality, and quantification of B-cell viral load could be useful to determine prognostic factors for response and long-term outcome. Although monoclonality is a factor predictive of lower survival [13], there is still no information about differences in response rate to rituximab.

Our data indicate that a larger prospective trial to test the association of rituximab and rapamycin in PTLD is warranted.

References

1. Cook RC, Connors JM, Gascoyne RD, Fradet G, Levy RD (1999) Treatment of post-transplant lymphoproliferative disease with rituximab monoclonal antibody after lung transplantation. Lancet 354:1698–1699
2. Dror Y, Greenberg M, Taylor G, Superina R, Hébert D, West L, Connolly B, Sena L, Allen U, Weitzman S (1999) Lymphoproliferative disorders after organ transplantation in children. Transplantation 67:990–998
3. Dumont FJ, Staruch MJ, Grammer T, Blenis J, Kastner CA, Rupprecht KM (1995) Dominant mutations confer resistance to the immunosuppressant, rapamycin, in variants of a T cell lymphoma. Cell Immunol 163:70–79
4. Faller DV, Mentzer SJ, Perrine SP (2001) Induction of the Epstein-Barr thymidine kinase gene with concomitant nucleoside antivirals as a therapeutic strategy for Epstein-Barr virus associated malignancies. Curr Opin Oncol 13:360–367
5. Faye A, Van Den Abeele T, Peuchmaur M, Mathieu-Boue A, Vilmer E (1998) Anti-CD20 monoclonal antibody for post-transplant lymphoproliferative disorders. Lancet 352:1285
6. Fischer A, Blanche S, Le Bidois J, Bordigoni P, Garnier JL, Niaudet P, Morinet F, Le Deist F, Fischer AM, Griscelli C (1991) Anti-B-cell monoclonal antibodies in the treatment of severe B-cell lymphoproliferative syndrome following bone marrow and organ transplantation. N Engl J Med 324:1451
7. Grillo-Lopez AJ (2000) Rituximab: an insider's historical perspective. Semin Oncol 27:9–16
8. Grillo-Lopez AJ, Lynch J, Corffier B, Connors JM, Swinnen LJ (1999) Rituximab therapy of lymphoproliferative disorders in immunosuppressed patients. Abstract 662. VII International Conference on Malignant Lymphoma. Lugano, 2–5 June
9. Hanto DW, Frizzera G, Gajl-Peczalska K, Balfour HH, Simmons RL, Najarian JS (1985) Acyclovir therapy of Epstein-Barr virus induced post-transplant lymphoproliferative diseases. Transplant Proc 17:89–92
10. Harris NL, Ferry JA, Swerdlow SH (1997) Posttransplant lymphoproliferative disorders (PTLD): summary of Society for Hematopathology Workshop. Semin Diagn Pathol 14:8–14
11. Knowles DM, Cesarman E, Chadburn A (1995) Correlative morphologic and molecular genetic analysis demonstrate three distinct categories of posttransplantation lymphoproliferative disorders. Blood 85:552–558
12. Kuehnle I, Huls MH, Liu Z, Semmelmann M, Krance RA, Brenner MK (2000) CD20 monoclonal antibody (rituximab) for therapy of Epstein-Barr virus lymphoma after hemopoietic stem-cell transplantation. Blood 95:1502–1505
13. Leblond V, Dhedin N, Bruneel MFM, Choquet S, Hermine O, Porcher R, Quoc SN, Davi F, Charlotte F, Dorent R, Barrou B, Vernant JP, Raphael M, Levy V (2001) Identification of prognostic factors in 61 patients with post-transplantation lymphoproliferative disorders. J Clin Oncol 19:772–778
14. Majewski M, Korecka M, Kossev P, Li S, Goldman J, Moore J, Silberstein LE, Nowel PC, Schuler W, Shaw LM, Wask MA (2000) The immunosuppressive macrolide RAD inhibits growth of human Epstein-Barr virus-transformed B lymphocytes in vitro and in vivo: a potential approach to prevention and treatment of posttransplant lymphoproliferative disorders. Proc Natl Acad Sci 97:4285–4290
15. Milpied N, Antoine C, Garnier JL, Parquet N, Vasseur B, Bouscary D, Carret AS, Faye A, Quartier P, Bourbigot B, Bourquard P, Hurault de Ligny B, Reguerre Y, Stoppa AM, Dubief F, Mathieu-Boué A, Leblond V (2000) Humanized anti-CD20 monoclonal antibody (rituximab) in B posttransplant lymphoproliferative disorders: a retrospective analysis of 32 patients. Ann Oncol [Suppl 1]:113
16. Muthukkumar S, Ramesh TM, Bondada S (1995) Rapamycin, a potent immunosuppressive drug, causes programmed cell death in B lymphoma cells. Transplantation 60:264–270
17. Niedermeier J, Hoffmeyer F, Hertenstein B, Hoeper MM, Fabel H (2000) Treatment of lymphoproliferative disease with rituximab. Lancet 355:499
18. O'Dwyer ME, Launder T, Rabkin JM, Nichols CR (2000) Successful treatment of aggressive post transplant lymphoproliferative disorder using rituximab. Leuk Lymphoma 39:411–419
19. Oertel SHK, Anagnostopoulos I, Bechstein WO, Liehr H, Riess HB (2000) Treatment of posttransplant lymphoproliferative disorder with the anti-CD20 monoclonal antibody rituximab alone in an adult after liver transplantation. Transplantation 69:430–432
20. Paya CV, Fung JJ, Nalesnik MA, Kieff E, Green M, Gores G, Habermannn TM, Wiesner RH, Swinnen LJ, Woodle ES, Bromberg JS (1999) Epstein-Barr virus-induced posttransplant lymphoproliferative disorders. Transplantation 68:1517–1525
21. Sacchi S, Federico M, Dastoli G, Fiorani C, Vinci G, Clo V, Casolari B (2001) Treatment of B-cell non-Hodgkin's lymphoma with anti CD 20 monoclonal antibody rituximab. Crit Rev Oncol Hematol 37:13–25

22. Seghal SN, Camardo JS, Scarola JA, Maida BT (1995) Rapamycin (sirolimus, rapamune). Curr Opin Nephrol Hypertens 4:482–487
23. Shan D, Ledbetter JA, Press OW (2000) Signaling events involved in anti-CD20-induced apoptosis of malignant human B cells. Cancer Immunol Immunother 48:673–683
24. Shapiro RS, Chauvenet A, McGuire W, Pearson A, Craft AW, McGlave P, Filipovich A (1988) Treatment of B-cell lymphoproliferative disorders with interferon-αand intravenous gamma-globulin. N Engl J Med 318:1334
25. Shapiro R, Nalesnik M, McCauley J, Fedorek S, Jordan ML, Scantlebury VP, Jain A, Vivas C, Ellis D, Lombardozzi-Lane S, Randhawa P, Johnston J, Hakala TR, Simmons RL, Fung JJ, Starzl TE (1999). Posttransplant lymphoproliferative disorders in adult and pediatric renal transplant patients receiving tacrolimus-based immunosuppression. Transplantation 68:1851–1854
26. Shi Y, Frankel A, Radvanyi LG, Penn LZ, Miller RG, Mills GB (1995) Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro. Cancer Res 55:1982–1988
27. Skoda-Smith S, Douglas VK, Mehta P, Graham-Pole J, Wingard JR (2001) Treatment of post-transplant lymphoproliferative disease with induction chemotherapy followed by haploidentical peripheral blood stem cell transplantation and rituximab. Bone Marrow Transplant 27:329–332
28. Starzl TE (1968) Five years' experience in renal transplantation with immunosuppressive drugs: survival, function, complications and the role of lymphocyte depletion by thoracic duct fistula (in discussion of Murray JE, Wilson RE, Tylney NL). Ann Surg 168:416–435
29. Starzl TE, Penn I, Halgrimson CG (1970) Immunosuppression and malignant neoplasms. N Engl J Med 283:934–937
30. Younes BS, McDiarmid SV, Martin MG, Vargas JH, Gross JA, Busutt RW (2000) The effect of immunosuppression on posttransplant lymphoproliferative disease in pediatric liver transplant patients. Transplantation 70:94–99