

Original article

Prolonged remission after a single course of rituximab in two young patients with severe refractory immune thrombocytopenia associated with systemic, lupus-like autoimmune disease.

Ricardo Russo, MD, Adriana Roy, Jorge Rossi, PhD, María Katsicas, MD

Service of Immunology, Hospital de Pediatría "Prof. Dr. Juan P. Garrahan"
Pichincha 1880, Buenos Aires (1245), Argentina

Contact:

Ricardo A. G. Russo, MD
Principal Physician, Service of Immunology
Hospital de Pediatría "Prof. Dr. Juan P. Garrahan"
Pichincha 1880, Buenos Aires (1245), Argentina
Tel: +54-11-4308-4300
Fax: +54-11-4308-5325
Email: russo@garrahan.gov.ar

Abstract

Two 15 year-old girls with severe, refractory thrombocytopenia associated with systemic autoimmune disease were treated with rituximab at 375 mg/m² per dose weekly for 4 weeks. Premedication with diphenhydramine 0.5 mg/Kg and hydrocortisone 1 mg/Kg was used. Methotrexate 7.5 mg/m²/week and methylprednisone 2 mg/kg/day were also used as combined medications. Rapid decrease in peripheral B cells was accompanied by a sharp increase in the platelet count. Other features of the multiorgan disease (autoimmune hemolytic anemia, rash, arthritis) also improved dramatically; titers of different autoantibodies decreased significantly. The corticosteroid dosage could be tapered in both patients with no relapses. Sustained improvement has lasted for over 18 months. Despite the prolonged B cell depletion (over a year-long), serum immunoglobulin levels did not decrease substantially, and the patients did not need any replacement therapy. B cell depletion appears to be a safe and efficacious therapy for severe, refractory thrombocytopenia in the setting of a systemic autoimmune disease.

Introduction

Rituximab is a new therapeutic monoclonal antibody that targets the CD20 antigen expressed on mature B cells [1]. Originally licensed for the treatment of B-cell lymphoma, it has been successfully used in the treatment of various autoimmune diseases, both systemic and organ-specific. In particular, a satisfactory response to rituximab has been reported in patients with childhood autoimmune hemolytic anemia, immune thrombocytopenia, and systemic lupus erythematosus [2-6]. In these diseases, the likely mechanism of action of rituximab is the elimination of cells producing anti-platelet and anti-erythrocyte antibodies, among others. We report

the use of rituximab in two adolescents with severe thrombocytopenia associated with systemic autoimmune disease.

Case reports

Case 1: The first patient is a 15-year-old girl with an autoimmune disease characterized by thyroiditis, arthritis, and insulin resistance. She presented with a high titer ANA (1:1000) and antimicrosomal antibodies. Anti-DNA and anti-ENA antibodies were negative. One year after disease onset, she developed chronic thrombocytopenia that precipitated frequent severe haemorrhage in different organs (uterine, gingival, and cutaneous). Her hematological disease was refractory to treatment with high dose methylprednisone (2 mg/kg/day), azathioprine (2 mg/kg/day for 8 months), two courses of IV gammaglobulin (2 g/kg each), and splenectomy. Her platelet count was persistently low at <1000 K/μL x 10⁹/L.
Case 2: The second patient is a 15-year-old girl with an undifferentiated systemic autoimmune disease. She presented with a nonspecific cutaneous rash, diffuse abdominal pain, myalgia, fever, positive ANA (1:1000), positive anticardiolipin antibodies (13 IgG phospholipid (gpl) units, 50 IgM phospholipid (mpl) units) and autoimmune haemolytic anaemia. Anti-DNA and anti-ENA antibodies were negative. She was treated with methylprednisolone 2 mg /kg/day for six weeks with a poor response; rash, abdominal pain, and hemolytic anaemia persisted. Two months after disease onset she experienced severe multiorgan (CNS, retinal, uterine, pulmonary) hemorrhage secondary to thrombocytopenia (platelet count<1000 K/μl) that was refractory to treatment with high dose methylprednisolone (1 g/day x 3 days) and IV gammaglobulin (2 g/kg). She was admitted to ICU and received several packed red blood cell transfusions.

After written informed consent was obtained from the patients' parents, rituximab was administered at 375 mg/m² per dose weekly for 4 weeks. Premedication with diphenhydramine 0.5 mg/kg and hydrocortisone 1 mg/kg was used. Rituximab was diluted in 250 ml of 5% dextrose, and it was infused over a period of 4–6 hours. Both patients also received initially methotrexate (MTX) 7.5 mg/week and methylprednisone 2 mg/Kg/day concomitantly with rituximab. MTX was discontinued 3 months after therapy with rituximab, and steroid doses were progressively tapered after the first month of therapy.

In both girls rituximab treatment quickly reversed the thrombocytopenia (Figure 1A).

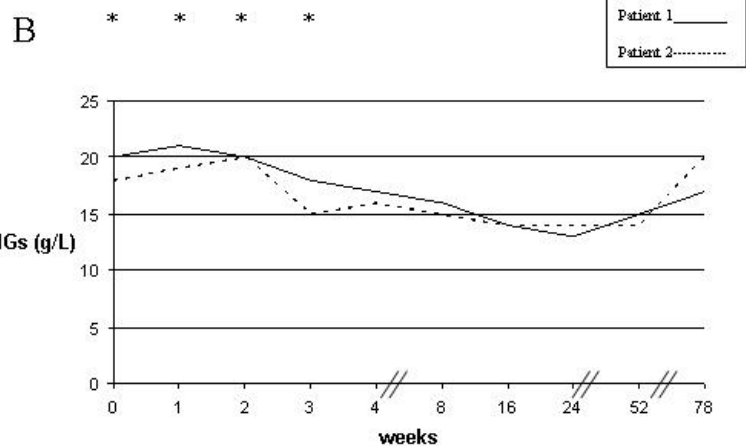
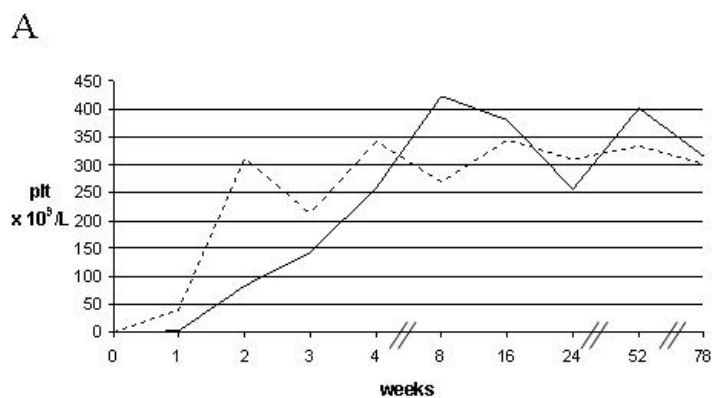


Figure 1 (A & B): platelet count (A) and serum immunoglobulin levels (IGs) (B) in patients 1 and 2. * = anti-CD20 infusions.

The first patient's platelet count reached normal levels (380,000 K/ μ L) after the third infusion. Eighteen months after treatment with rituximab she is asymptomatic, she has experienced no new hemorrhage, and she has had no infections. Serum immunoglobulin levels and platelet counts have been normal; her peripheral blood CD19+ B cells were first detectable at week 60. Antimicrobial antibodies and ANA were first noted to be negative three months after rituximab therapy and they have remained negative. Peripheral blood CD19 + B cells were first detected on week 50 after treatment (9% of lymphocytes). She is currently receiving oral methylprednisone at 4 mg/day.

The platelet count of patient #2 rose to 250,000 x K/ μ L six days after the first infusion. She has had no symptoms of her systemic disease, no recurrence of hemorrhage, nor any infections during the 18 months that followed treatment with rituximab. She is currently off steroids, and both serum immunoglobulin level (1400-1600 mg/dL) and platelet count (250,000-310,000 x K/ μ L)

have remained in the normal range. Her anticardiolipin and antinuclear antibodies are negative.

In both girls, extrahematological systemic features (such as arthritis in the first patient, and cutaneous rash in the second one) also remitted completely after rituximab infusion, and no active inflammation has been evident after eighteen months of follow-up. Serum anti-pneumococcal and anti-tetanus antibodies have remained within the protective range.

Discussion

These cases illustrate the effectiveness of rituximab in refractory immune thrombocytopenia in the setting of systemic, lupus-like disease. In both patients, platelet counts reached normal levels by the third infusion of rituximab, following the B cell depletion observed during the first weeks. Improvement has been sustained for over 18 months, without the need of further immunosuppressive therapy beyond the single initial course of rituximab. Not only did platelet counts improve in these girls, but also extrahematological, systemic features of autoimmune disease, both clinical and serological, showed clear signs of improvement. Moreover, an additional measure of clinical efficacy was that the corticosteroid dosage could be tapered without occurrence of relapses in either patient.

The serum titers of different autoantibodies changed significantly in both patients. B cell depletion was paralleled by a marked decrease in the titers of ANA, anticardiolipin and anti-thyroid antibodies. Unfortunately, anti-platelet antibodies, likely pathogenetic in these cases, could not be investigated in our patients. Despite the fact that this treatment was associated with a massive reduction of circulating B cells, there was only a slight decrease in serum gammaglobulin levels during the follow-up period (Figure 1B). This more pronounced fall in serum autoantibodies levels as compared to the total serum immunoglobulin levels in patients treated with rituximab has already been observed by other investigators. [7-9] It is likely that other immunoglobulin-producing cells such as plasma cells, which are not targeted by rituximab, continue secreting antibodies. In our patients, B cell depletion in the peripheral blood occurred within days after the first anti-CD20 infusion and B cells counts recovered only by the 18th month after rituximab therapy. Other investigators have found that the duration of B cell depletion after rituximab therapy to be widely variable. [8-9]

A single course of rituximab has proved to be effective in the long term in the treatment of chronic immune thrombocytopenia and haemolytic anaemia in the absence of systemic autoimmune disease. Zaja et al. successfully treated six adult patients with either autoimmune hemolytic anemia or chronic thrombocytopenia previously refractory to conventional treatments with weekly infusions of rituximab, 375 mg/m², for 4 weeks, without the addition of immunosuppressors. [10] However, the response duration was less than 18 weeks in all patients but one. Shanafelt et al. treated 14 adult patients with autoimmune cytopenias with at least

one dose of rituximab at 375 mg/m², alone and in combination with different immunosuppressive medications. [11] Complete hematological remission was achieved in 40% of their patients, and response duration varied between 4 and 13 months. Quartier et al used the same therapeutic approach in six children with chronic autoimmune hemolytic anemia; Sustained, prolonged remission was observed but serum immunoglobulins levels were significantly decreased and IVIG replacement was used in five patients. [12]

In our cases, not only did the hematological features steadily improve, but also systemic inflammatory features of their disease (rashes, arthritis and other clinical features) also experienced a marked and prolonged improvement. Rituximab has been used in the treatment of adult patients with systemic autoimmune diseases, such as SLE and rheumatoid arthritis [6, 8-9, 13-15], while published experiences in pediatric patients are limited to isolated reports. [4-5] Binstadt et al reported the efficacy of rituximab in the treatment of four juvenile patients with refractory, multisystem autoimmune disease characterized by a predominant central nervous system involvement. [5] These authors utilized the same rituximab dosage in their patients that we used in our patients. Also, in some of the patients of Binstadt et al., cyclophosphamide had been used before the therapy with rituximab was started. Surprisingly, the levels of total serum immunoglobulins dropped markedly in their cohort, making IVIG replacement necessary in three of their cases.

Concomitant corticosteroid therapy was given to our patients. Although it was rapidly tapered after the four courses of rituximab infusion, combination therapy may have contributed to the efficacy of the treatment in our group. High dose steroids and cyclophosphamide have been combined with rituximab in the successful treatment of SLE and rheumatoid arthritis in different trials. [8-9, 13-15]

No infusion-related events or side-effects occurred in our two patients. It is reassuring that at eighteen months after treatment with anti-CD20 antibodies, these patients have required no supplementary IV gammaglobulin, have not had any serious infections, and maintained protective antibody titres to tetanus and pneumococcus. Several reports have also shown that this dose of rituximab appeared to be safe and well-tolerated in different cohorts of patients with juvenile [4] and adult SLE. [8-9]

Conclusion

Combination therapy utilizing rituximab may be an efficacious and safe treatment for patients with refractory autoimmune thrombocytopenia in the setting of a systemic autoimmune condition. Improvement may be sustained and exceed the duration of B cell depletion. A prospective controlled trial is needed to more fully assess the efficacy and tolerability of rituximab as an early, steroid sparing therapy in refractory cases of autoimmune diseases.

References

1. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood* 1994;83:435-45.
2. Pusiol A, Cesaro S, Nocerino A, Picco G, Zanesco L, Bisogno G. Successful treatment with the monoclonal antibody rituximab in two children with refractory autoimmune thrombocytopenia. *Eur J Pediatr*. 2004;163:305-7.
3. Cooper N, Stasi R, Cunningham-Rundles S, Feuerstein MA, Leonard JP, Amadori S, et al. The efficacy and safety of B-cell depletion with anti-CD20 monoclonal antibody in adults with chronic autoimmune thrombocytopenic purpura. *Br J Haematol*. 2004;125:232-39.
4. Ten Cate R, Smiers FJ, Bredius RG, Lankester AC, Van Suijlekom-Smit LW, Hizinga TW, et al. Anti-CD20 monoclonal antibody (rituximab) for refractory autoimmune thrombocytopenia in a girl with systemic lupus erythematosus. *Rheumatology* 2004;43:244.
5. Binstadt BA, Caldas AM, Turvey SE, Stone KD, Weinstein HJ, Jackson J, et al. Rituximab therapy for multisystem autoimmune diseases in pediatric patients. *J Pediatr*. 2003;143:598-604.
6. Weide R, Heymanns J, Pandorf A, Koppler H. Successful long-term treatment of systemic lupus erythematosus with rituximab maintenance therapy. *Lupus* 2003;12:779-782.
7. Cambridge G, Leandro MJ, Edwards JCW, Ehrenstein MR, Salden M, Bodman-Smith M, et al. Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis. *Arthritis Rheum*. 2003;48:2146-2154.
8. Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F, Arend LJ, et al. B cell depletion as a novel treatment for systemic lupus erythematosus: a Phase I/II dose-escalation trial of rituximab. *Arthritis Rheum*. 2004;50:2580-9.
9. Sfikakis PP, Boletis JN, Lionaki S, Vigiaklis V, Fragiadaki KG, Iniotaki A, et al. Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 Ligand. An open-label trial. *Arthritis Rheum*. 2005;52:501-513.
10. Zaja F, Iacona I, Masolini P, Russo D, Sperotto A, Prosdocimo S, Patriarca F, et al. B-cell depletion with rituximab as treatment for immune hemolytic anemia and chronic thrombocytopenia. *Haematologica*. 2002;87:336.
11. Shanafelt TD, Madueme HL, Wolf RC, Tefferi A. Rituximab for immune cytopenia in adults: idiopathic thrombocytopenic purpura, autoimmune haemolytic anemia, and Evan's syndrome. *Mayo Clin Proc*. 2003;78:1340-1346.
12. Quartier P, Brethon B, Philippet P, Landman-Parker K, Le Deist F, Fischer A. Treatment of childhood autoimmune haemolytic anemia with rituximab. *Lancet* 2001;358:1511-1513.
13. Silverman GJ, Weissman S. Rituximab therapy and autoimmune disorders. Prospects for anti-B cell therapy. *Arthritis Rheum*. 2003;48: 1484-1492.
14. Edwards JCW, Szczepansky L, Szechinsky J, Filipowicz-Sosnowska A, Emery P, Close DR, et al. Efficacy of B-cell targeted therapy with rituximab in patients with rheumatoid arthritis. *N Engl J Med*. 2004;350:2572-2581.
15. Leandro MJ, Edwards JCW, Cambridge G. Clinical outcome in 22 Patients with rheumatoid arthritis treated with B lymphocyte depletion. *Ann Rheum Dis*. 2002;61:883-888.