Long-term remission induced by low-dose rituximab for relapsed and refractory thrombotic thrombocytopenic purpura: A report of two cases

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Abstract. Thrombotic thrombocytopenic purpura (TTP) is acquired in the majority of cases. Traditional therapy consists of plasma exchange (PEX), as well as the administration of certain immunosuppressive agents including steroids. A standard dose of rituximab (RTX) at 375 mg/m^2 weekly for 4 consecutive weeks was recently demonstrated to have significant activity in patients with acquired TTP. To date, clinicians have limited experience using low-dose RTX. In the present study, 2 patients were treated with low-dose RTX at 100 mg weekly for 4 consecutive weeks as a salvage therapy following failure to respond to PEX and other immunosuppressive agents. Prior to RTX therapy, the patients had severely deficient ADAMTS13 activity and detectable anti-ADAMTS13 inhibitors. The patients achieved complete remission and presented long-term stabilization during follow-up. Repeated detection during follow-up demonstrated that the patients had 100% ADAMTS13 activity and undetectable anti-ADAMTS13 antibodies. Although further investigation in a prospective clinical trial is required, the use of low-dose RTX seems to be as effective as a standard dose for patients with relapsing or refractory acquired TTP.

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare blood disorder, which is acquired in the majority of cases. TTP is characterized by thrombotic microangiopathy, which may lead to microangiopathic hemolytic anemia and thrombocytopenia with or without neurological symptoms, kidney damage and fever (1). This disease is further divided into idiopathic TTP and secondary TTP, with the idiopathic type being the most common form of acquired TTP. In acquired TTP, antibodies against ADAMTS13 are detected in the patients' serum, leading to deficient ADAMTS13 enzyme activity (generally <5%). ADAMTS13 is a metalloproteinase that is responsible for cleaving large multimers of von Willebrand factor (vWF) into smaller units. Due to decreased ADAMTS13 activity in TTP, the plasma vWF multimers may not be eliminated, and thus thrombosis occurs with platelet accumulation (2).

The current international standard treatment for TTP is plasma exchange (PEX), which reduces mortality by ≤90% (1). However, relapse is common in patients only receiving PEX. Hence, patients with acquired TTP receive additional immunosuppressive therapy, such as glucocorticoids, which are able to suppress ADAMTS13 autoantibodies and reduce pathogenic cytokine levels (3). However, relapse rate of patients with TTP remains at 30-50%, and the first relapse usually occurs within 1 year following treatment. Relapse of TTP is the primary cause of mortality and thrombosis syndrome. In order to reduce TTP recurrence, other immunosuppressive agents such as cyclosporine A, vincristine, cyclophosphamide, and rituximab (RTX) may be used.

RTX is a chimeric mouse-human monoclonal antibody against the B-lymphocyte antigen, CD20, which is primarily detected on the surface of B-cells. A standard dose of RTX (a commonly-used dose in B-cell lymphoma is 375 mg/m^2/week, continuously for 4 weeks) was initially used for the treatment of autoimmune diseases and immune TTP (a type of secondary TTP), and has resulted in encouraging results (4,5). Zaja *et al* (6) conducted a prospective clinical trial attempting to treat relapsed and newly-diagnosed acquired ITP patients with a low-dose of RTX (100 mg weekly, for 4 weeks). The authors concluded that low-dose RTX administration could achieve a similar efficacy as treatment with the standard dose (6). With acquired TTP, abnormal B-lymphocytes produce ADAMTS13 autoantibodies. The antibody was first successfully used to treat non-Hodgkin lymphomas of B-cell origin; however, there is evidence for its efficacy in the treatment of other hematological or autoimmune diseases like autoimmune hemolytic anaemia (7) or chronic idiopathic thrombocytopenia (ITP). The treatment mechanism of TTP by RTX is similar to that in ITP; the production of

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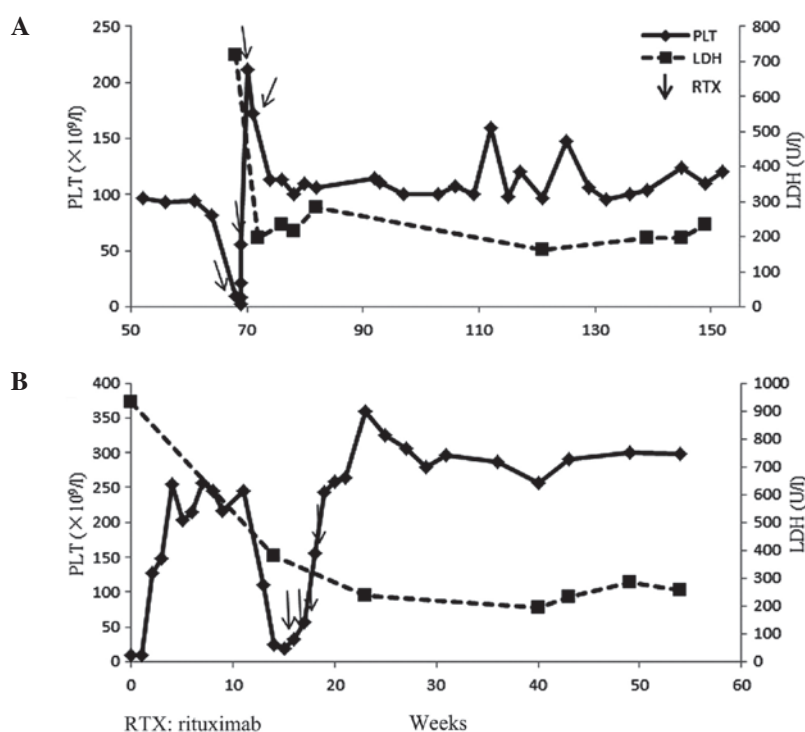E-mail: zjuhongyantong@163.com

Figure 1. PLT (x10⁹/l) and LDH levels (U/l), indicating the response to treatment in (A) case 1 and (B) case 2. PLT, platelet count; LDH, lactate dehydrogenase.

autoantibodies and the mitigation of the activity of B lymphocytes antigen-activated T lymphocytes. Fakhouri *et al* (8) and Scully *et al* (9) prospectively investigated the administration of a standard dose of RTX (375 mg/m^2/week, continuously for 4 weeks) for the treatment of relapsed and newly-diagnosed TTP patients. They observed that the majority of cases experienced increased ADAMTS13 activity and a decreased number of ADAMTS13 antibodies, while the platelet count in 68% of patients increased to >50x10⁹/l before commencing the second RTX infusion week (8,9). Furthermore, a significant reduction in the relapse rate was observed, and the majority of patients maintained a longer remission following RTX therapy. Multiple clinical studies concurrently demonstrated that a standard dose of RTX is effective in the treatment of TTP (10-12). Based on the successful application of standard-dose RTX for TTP treatment and low-dose RTX for ITP treatment in previous studies, the present study investigated the use of low-dose RTX for the treatment of relapsed or refractory TTP. In the present study, 2 successfully treated cases of TTP were presented. Written informed consent was obtained from the patients.

Case report

Case 1. A 38-year-old male presented at the First Affiliated Hospital of Zhejiang University (Hangzhou, China) with petechia and ecchymosis on the entire body in August 2011. Laboratory tests revealed a normal hemoglobin level (14.5 g/dl), a reduced platelet count (8x10⁹/l), an elevated lactate dehydrogenase (LDH) level (580 U/l) and erythrocyte count of 2,073/μl in the urine. An ultrasound of the urinary system was normal and bone marrow smears revealed megakaryocytic hyperplasia. The patient then developed abnormal psychological symptoms; however, a computed tomography (CT) scan of

the head revealed no abnormalities. The hemoglobin level was reduced to 9.7 g/dl, while the platelet count remained at 8x10⁹/l. A further blood test indicated that the ADAMTS13 activity was deficient, with the presence of circulating ADAMTS13 inhibitor. Subsequent to excluding secondary causes, the patient was diagnosed with TTP.

The patient received 9 sessions of PEX, along with administration of oral cyclosporine A (CsA; 5 mg/kg, total 300 mg). PEX and dexamethasone (10 mg/day) were immediately administered. Due to plasma shortage, PEX was administered at least once every 2 days (a total of 6 PEXs). After 1 week, 150 mg CsA was administered every 12 h, and the period of PEX was once a week when PLT increased to normal (a total of 3 PEXs). Blood cell count was evaluated twice a week. If blood cell count remained stable, dexamethasone dose was gradually tapered (one or two tablets were reduced per 2 weeks) until stop, and CsA was reduced by 50 mg/week to maintenance therapy dose of 50 mg/day. CsA was discontinued at relapse after 1 year and changed to dexamethasone and PEXs, and RTX. The platelet count of the patient reached >100x10⁹/l, and the CsA administration was gradually tapered until it was discontinued. On week 68 after first admission, the patient presented with hematuria and skin petechia. Laboratory tests revealed an extremely low platelet count of 4x10⁹/l, a hemoglobin level of 13.3 g/dl, a reticulocyte count of 3.3% and an LDH level of 836 U/l. Repeated detection demonstrated deficient ADAMTS13 activity and detectable levels of anti-ADAMTS13 inhibitor. Therefore, a diagnosis of refractory TTP was concluded. The patient was administered a low-dose of RTX, at 100 mg/week, continuously for 4 weeks. In the first week of RTX treatment, the patient received 2 PEX sessions. Concurrently, the patient received 40 mg/day methylprednisolone, which continued for ~3 months following the initiation of the RTX treatment, and the dose was gradually

tapered until discontinuation. The treatment was well-tolerated with no side-effects. Following the first week of RTX treatment, the patient's platelet count increased to 220x10⁹/l and the LDH level returned to the normal levels. During the follow-up, repeated detection demonstrated 100% ADAMTS13 activity and undetectable levels of anti-ADAMTS13 antibodies. The patient remained asymptomatic with a normal platelet count in August 2015 (Fig. 1A).

Case 2. A 34-year-old female presented at the First Affiliated Hospital of Zhejiang University with a sudden headache, nausea and vomiting associated with fever and an altered mental status in May 2012. A central nervous system examination was unremarkable and a CT scan of the head revealed no abnormalities. Laboratory tests demonstrated the following: A reduced platelet count compared with normal values ($6x10^9$/l); reduced hemoglobin level (8 g/dl); an elevated LDH level (932 U/l); total bilirubin, 2.4 mg/dl; direct bilirubin, 1.2 mg/dl; plasma free hemoglobin, 1.55 mg/dl; and erythrocyte count in urine, 63.8/μl. Bone marrow smears revealed erythroid hyperplasia. A peripheral blood smear showed poikilocytosis and evident erythrocyte debris. Further detection revealed deficient ADAMTS13 activity, detectable anti-ADAMTS13 inhibitor levels and a reticulocyte count of 22.5%. Antinuclear antibody titers, immunoglobulin, thyroid function, tumor markers (AFP, CEA, CA199, CA153 and CA155) and a Coombs' test were negative, and thus a diagnosis of ITP was concluded. After the patient was treated with 2 PEX sessions, the hemoglobin level increased to 7.4 g/dl, with a reticulocyte count of 16.3% and a platelet count of $128x10^9$/l. A prednisone dose of 30 mg/day was orally administered for ~3 months and then gradually tapered until discontinuation. On week 14, the patient's platelet count decreased again to $25x10^9$/l. A plasma transfusion and PEX were administered and the prednisone dose was adjusted to 60 mg/day, but the platelet count did not improve significantly. On week 15, the patient was administered a low intravenous dose of RTX, at 100 mg/week, continuously for 4 weeks. The treatment was well-tolerated without any side-effects. The platelet count increased to the normal level following the second week of RTX treatment. The patient recovered (hemoglobin level, 11.2 g/dl; platelet count, $235x10^9$/l) in June 2014. At week 49 following treatment, the patient became pregnant and successfully delivered a healthy child, without any hematological abnormalities. The patient was in good condition at the 23-month follow-up. Regular testing demonstrated that her platelet count, LDH level and serum ADAMTS13 activity were maintained within the normal levels (Fig. 1B). Regular testing demonstrated that the patient's platelet count, LDH level and serum ADAMTS13 activity remained within the normal levels in August 2015.

Discussion

In the majority of cases, TTP is caused by auto-antibodies that inhibit the vWF multimer-cleaving enzyme, ADAMTS13. Prospective studies have demonstrated that a standard dose of RTX is effective for the treatment of immune TTP, if patients failed to respond to daily PEX and steroids, as well as for the treatment of relapsed acute ITP (8,9). The British Committee for Standards in Heamatology published guidelines regarding

the diagnosis and management of TTP and recommended that patients with refractory or relapsing immune-mediated TTP should be administered RTX, typically at a dose of 375 mg/m² weekly for 4 weeks (13). However, to date, clinicians have limited experience using low-dose RTX for the treatment of patients with acquired TTP.

The 2 refractory and relapsed TTP cases presented in the current study were treated with low-dose RTX using a dose of 100 mg per week for 4 consecutive weeks. The 2 patients obtained favorable outcomes and achieved a sustained, long-term remission. As of this report, 1 patient achieved permanent remission for 23 months, while the other patient, who was refractory to PEX, steroids and CsA, was also in remission for 19 months. Recently, Pequeño-Luévano *et al* (14) reported the use of low-dose RTX (100 mg/day, continuously for 7 days) as a first-line therapy at the same time as PEX treatment in 3 ITP cases, and as a salvage therapy for a relapsing case. With this treatment, all 4 patients achieved complete remission, were asymptomatic as of the report and had achieved a complete response duration of 8-22 months (14). Coincidentally, similar to the observations of the previous study, the present case report illustrated that low-dose RTX treatment may be an effective alternative for certain acute acquired TTP cases, particularly for patients with relapsed and refractory disease.

The mechanism though which RTX functions in the treatment of TTP is similar to its function in ITP. It works mainly by eliminating activated CD20⁺ B-lymphocytes, increasing the number of regulatory T (Treg) cells, and improving the function of the Treg cells. Regarding the effectiveness of low-dose RTX, possible mechanisms may involve the small amount of abnormally activated B-lymphocytes in TTP, as opposed to clonal B-lymphocytes in malignant lymphoma; thus, a lower RTX dose may eliminate the abnormally activated B-lymphocytes. In addition, certain studies have demonstrated that after the first week of RTX treatment, peripheral blood CD20⁺ cells had almost disappeared in ITP patients (15,16). Therefore, for autoimmune diseases, the current authors hypothesized that RTX may not require a dose as large as that used in B-cell lymphoma. Furthermore, the successful treatment of ITP patients involving low-dose RTX also demonstrates the effectiveness of low-dose RTX for autoimmune diseases. Under the premise of ensuring efficacy, the lower the RTX dose, the lower the side-effect rate will be.

In conclusion, the present study described the successful treatment of 2 cases using low-dose RTX for relapsed and refractory TTP, and the results in the two cases were independent of the PEX treatment. However, numerous questions remain to be answered, including which RTX dose is the most suitable for the treatment of TTP. Furthermore, the frequency and timing of RTX remain to be investigated. Therefore, further prospective clinical investigation is required on the use of low-dose RTX for the treatment of TTP.

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