Original Article
Efficiency of treatment with rituximab in platelet transfusion refractoriness: a study of 7 cases

Wenbin Liu*, Dijiong Wu, Tonglin Hu, Baodong Ye*

*Department of Hematology, First Hospital Affiliated to Zhejiang Chinese Medical University, Hangzhou, China. *Equal contributors.*

Abstract: Objective: The purpose of our study was to evaluate the efficacy and safety of rituximab in treatment of immune PR. Methods: We retrospective analysis 7 paitents (5 aplastic anemia, 2 myelodysplastic syndrome) with immune PR who received at least 3 weekly infusions of rituximab (375 mg/m^2). Results: All enrolled patients acquired improvement of platelets transfusion more than 2 months (CCI $\geq 4.5 \times 10^9$/L). We first found that there were 2 patterns of response to rituximab treatment in patients with immune PR, which the early but transient after the first rituximab administration and the late but continuous beginning to appear at 3 weeks from the start of treatment. Conclusion: Rituximab is a promising treatment in patients with immune PR and giving the opportunity and time for cure the disease.

Keywords: Rituximab, platelet refractoriness, corrected count increment, immune

Introduction

Platelet refractoriness (PR) can simply be defined as post-transfusion platelet increment that is less than expected [1], which is a life-threatening complication in patients with hematological disease. Platelet transfusion failure is a common phenomenon affecting from 7% to 34% of haematology patients, who are relied on platelet transfusion [2]. The causes of platelet refractoriness were mainly subdivided into immune and non immune causes. Immune causes include alloimmunization to HLA and/or platelet specific antigens due to prior exposure from pregnancy, transfusions and transplantation. Platelet antigens causing alloimmunization can be separated in two main groups, the human leukocyte antigen (HLA) system and the human platelet antigen (HPA) System [3], which the former is more common than the latter and is believed to be the primary cause of immune mediated platelet refractoriness. Moreover, the incidence of alloimmunization varies with different kinds of diseases. For example, HLA alloimmunization is more frequent in patients with aplasic anaemia than in patients with acute leukemia.

At present, the management of patients with HLA and/or HPA alloimmunization with no compatible donor may be very difficult. But only 50-60% of HLA selected transfusions produce satisfactory increments in alloimmunized patients and the response usually transient. Other management approaches for severe alloimmune refractoriness, such as high dose intravenous immunoglobulin (IV IgG), splenectomy and plasma exchange, have been shown to be ineffective [4]. Therefore, the management of PR still a great challenge.

Rituximab is a chimerical mouse/human monoclonal antibody directed against the CD20 determinant on B cell. It was initially developed for the treatment of B cell malignant lymphoma but has also been widely used in autoantibody mediated disorders, such as idiopathic thrombocytopenic purpura (ITP) [5], autoimmune hemolytic anemia (AIHA) [6], or thrombotic thrombocytopenic purpura (TTP) [7]. Case reports described it is promising results in PR patients [8, 9], but it is lack of sufficient evidence base data. We report the beneficial effect of rituximab in the treatment of a cohort of PR patients.

Table 1. Platelet refractory patients:clinical characteristics

Patients no.	Sex	Age	Diagnosis	Platlets supports (mos)	HLA/PA-IgG Screen	Complication
1	F	26	SAA	2.0	Anti-HLA+	HT
2	F	24	SAA	2.0	PA-IgG+/Anti-HLA+	HT/SLE
3	M	14	SAA	3.0	PA-IgG+	N
4	M	13	SAA	4.0	N	HB
5	F	46	CAA	2.0	PA-IgG+	HB
6	F	35	MDS	10.0	PA-IgG+	SS
7	F	23	MDS	4.0	N	N

N, no; SAA, severe aplastic anemia; CAA, Chronic aplastic anemia; MDS, Myelodysplastic syndrome; SLE, Systemic lupus erythematosus; SS, Sicca syndrome; HT, Hashimoto's thyroiditis; HB, Hepatitis B.

Materials and methods

The study was retrospective conducted between January 2004 and April 2014 in one center (The first affiliated hospital of Zhejiang Chinese Medical University). Appropriate consent was obtained from all patients.

Inclusion criteria

All the following criteria were required for inclusion: (1) age of at least 13 years; (2) significant bleeding symptoms and platelet $< 10 \times 10^9$/L; (3) no respond to random donor platelets transfusion and HLA matched platelets transfusion; (4) no respond to IV IgG therapy; (5) test HLA antibody and platelet associated IgG (PA-IgG).

Exclusion criteria

One of the following criteria was enough for exclusion in previous groups of patients: (1) serious infection; (2) palpable hepatosplenomegaly; (3) disseminated intravascular coagulation; (4) using chemotherapeutic agents at the time of entry into the study.

Treatment with rituximab

All patients received rituximab infusion over 6 to 8 hours at a dose of 375 mg/m^2 weekly either 4 cycles (patients 1, 2, 4 and 7), 3 cycles (patient 3, 5 and 6). The initial infusion rate was 50 mg/h, with a subsequent infusion rate increase up to 300 mg/h if no toxicity was seen. Patients received oral chlorphenamine 10 mg and iv dexamethasone 5 mg as premedication therapy. The patients who combined with Hepatitis B had to take Entecavir tablets more than 2 weeks before rituximab therapy.

Criteria for platelet response

The corrected count increment (CCI) is calculated from the corrected count increment (PI), the body surface area of the patient in square meters (BSA) and the dose of platelets transfused ($\times 10^{11}$) (PD) [10]: CCI = PI $\times$ BSA $\times$ PD^{-1}. CCI $\geq$ 4.5 $\times 10^9$/L is considered to be effectively responses to platelet transfusion after 20-24 hours; in contrast, CCI $< 4.5 \times 10^9$/L is considered to be ineffectively. A single apheresis of transfused platelet generally contains approximately 2.5 $\times 10^{11}$ platelets.

Results

Patient characteristics at inclusion

The clinical characteristics of the patients at the time of inclusion are summarized in **Table 1**. 7 patients were enrolled in the study, including 5 patients (patients 1 to 5) were aplastic anemia, the others were myelodysplastic syndrome (MDS). There were 2 males and 5 females, and the median age of 25.8 years (rang 13 to 46 years). At inclusion in the study, the patients have been depended on platelet transfusion at least 2 months (range 2 to 10 months). All patients suffered from skin petechiae, ecchymoses and non-traumatic bleeding, but one (patient 7) exhibited limited hemorrhage into the CNS. Generalized immunization, as judged by HLA antibody and platelet associated IgG (PA-IgG) screening, was detected positive in 5 patients (patients 1, 2, 3, 5 and 6).

In this series, Platelets from random donors were efficient in all enrolled patients after administration of rituximab and continued more than 2 months (CCI $\geq$ 4.5 $\times 10^9$/L). The bleeding symptoms of them had significantly relieved. Especially, the patient (patient 7) with cerebral hemorrhage was not left obvious neurologic sequelae because of undelayed treatment.

Table 2. Platelet transfusion responses after first rituximab therapy

Patients no.	The cycles of rituximab Administered (375 mg/m^2)	Response (CCI × 10^9/L) Pretreatment	Posttreatment	Following Therapy
1	4	1.57	15.07	ATG
2	4	1.58	13.46	ATG
3	3	2.43	12.38	ATG
4	4	1.12	17.35	ATG
5	3	2.98	22.61	Allo-HSCT
6	3	2.07	17.2	Allo-HSCT
7	4	1.33	26.59	Allo-HSCT

CCI, corrected count increment; ATG, antithymocyte globulin; allo-HSCT, allogeneic hematopoietic stem cell transplantation.

Response patterns to treatment

All patients with response a significant rise in platelet increment were observed early during the course of treatment, that usually immediately after first rituximab infusion, although the response was transient less than a week. On the other hand, the enrolled patients required sustained response in 3 weeks after first rituximab administration. The results obtained about platelet transfusion responses after first rituximab therapy are summarized in **Table 2**.

Tolerance

Rituximab appeared to be tolerated quite satisfactorily and no evidence of typical infusion related side effects, infectious complications or other late events were seen in any patients.

Follow-up

Follow up ranged from 2 months to 10 years. Following the effect of platelet transfusion was improved after rituximab therapy, 5 patients (patients 1 to 4) and 2 patients (patients 5 to 7) were received antithymocyte globulin (ATG) therapy and allogeneic hematopoietic stem cell transplantation (allo-HSCT) respectively. They were all successfully completed the therapeutic program as planned and out of the platelet transfusion dependence. Except patient 3, they have already fully recovered.

Discussion

Rituximab, an anti-CD20 monoclonal antibody, has proved efficiency in the treatment of CD20$^+$ lymphoproliferative disorders. It induces a B cell depletion that lasts 6 to 9 months with the recovery of a normal B cell count usually occurring 12 months after treatment [11]. The tolerance of rituximab is excellent. The efficacy of rituximab also has been confirmed for autoimmune diseases, especially in severe forms resistant to conventional immunosuppressive treatment.

Platelet transfusion therapy is the standard of care for thrombocytopenic patients with hematoglical disorders and could significantly decrease the morbidity and mortality in thrombocytopenic patients. But we are far from understanding the interplay of multiple factors that affect response to platelet transfusion. Several factors can affect the outcome of platelet transfusion, which mainly separate in immune and non immune causes [12]. Non-immune platelet destruction mainly caused by serious infection, palpable hepatosplenmegaly, disseminated intravascular coagulation and chemotherapeutic agents treatment is more common than immune mediated causes [13]. But in this study, all enrolled patients are ruled out the above factors.

At present platelet refractoriness remains a great challenge to manage patients who rely on platelet transfusion support. Whether HLA matched platelet or conventional approaches to the treatment of immune thrombocytopenia, the treatment effect is not satisfactory [14, 15]. Recently, there were reports showed that rituximab has been successfully used in platelet refractoriness, but the two reports both only single case.

We first report the 7 PR patients who treatment with rituximab. Firstly, our results confirm previous preliminary reports concerning the efficacy of rituximab in patients with immune PR. In the absence of any clear evidence supporting the efficacy of other therapeutic agents in this study, our data suggest that rituximab is a promising therapeutic agent in patients with immune PR.

Secondly, our results show that 2 patterns of response, early and late, to rituximab treatment

in patients with PR, which similar with previously reported rituximab treatment of ITP [16, 17]. We observed that the enrolled responders a significant rise in platelet increment after transfusion early during treatment, usually right after the first rituximab administration, but the effect always transient less than a week; the late response that stable continuous improve platelet transfusion beginning to appear at week 3 weeks from the start of treatment. With the limited data, we can only speculate the pathogenetic mechanism about the 2 patterns of response. It is possible that in early responders opsonized B cells block the macrophage system, which remains the mechanism of Fc receptor (FcR) blockade by opsonized red cells following anti-D immunoglobulin treatment [18]. The decreased production of antibodies accounts for the late and sustained response. In the late responders, the FcR blockade effect for some reason weakened. Otherwise, the patients with PR who following received conditioning regimen or ATG treatment may contribute to improve the effect of platelet transfusion [19]. However, the efficiency of rituximab in inducing the time of during remains to be evaluated in prospective controlled studies.

Thirdly, the improvement of platelet transfusion give the precious opportunity for the patients with PR to cure the diseases. In this series, 4 patients and 3 patients were received ATG and allo-HSCT therapy respectively, when the effect of platelet transfusion was improved after rituximab therapy. They all successfully completed the therapeutic program as planned, and they all became independent on the platelet transfusion. Six of them have been fully recovered at present; the patient (patient 4) who remains completely healthy has been more than 10 years. The patient (patient 3) who is not recovered now still has good response to platelets transfusion.

In addition, the tolerance of rituximab treatment was as good as reported previously in other autoimmune diseases. Despite these promising data, the effect of rituximab on long-term evolution of PR remains to be ascertained.

In summary, out data indicate that rituximab is an effective therapy in patients with PR and striving for the opportunity and time for cure the disease.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Baodong Ye, Department of Hematology, First Hospital Affiliated to Zhejiang Chinese Medical University, 54 Youdian Road, Hangzhou, China. Tel: +86-571-86620325; Fax: +86-571-86620322; E-mail: szyyblood@163.com

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