Efficacy of low-dose rituximab in combination with recombinant human thrombopoietin in treating ITP

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Abstract. – **OBJECTIVE:** This work aims to observe the efficacy and safety of low-dose rituximab in combination with recombinant human thrombopoietin in treating immune thrombocytopenia (ITP).

PATIENTS AND METHODS: Fourteen ITP patients were treated four times with 100 mg qw of rituximab in combination with 300 μg/kg/d ih recombinant human thrombopoietin (rhTPO) for 14 d. Platelet count in peripheral blood, serum immunoglobulin, and lymphocyte subgroups by flow cytometry were detected regularly both pre- and post-treatment.

RESULTS: Among the 14 patients, seven complete responses, six responses, and one no response were obtained, with an overall response of 93%.

CONCLUSIONS: Low-dose rituximab in combination with rhTPO is effective in treating ITP.

Key Words:
  Rituximab, Thrombopoietin, Thrombocytopenia, Immune, Primary.

Introduction

Adult primary immune thrombocytopenia (ITP), an acquired organ-specific autoimmune disease, is the most frequent hemorrhagic disease observed in clinical practice. ITP is characterized by thrombocytopenia due to excessive platelet destruction caused by autoantibodies against platelets generated in the human body. Despite traditional treatments, including glucocorticoids, immunoglobulins, splenectomy, and immunosuppression, no response has been observed in a small number of patients who manifest repeated thrombocytopenia and hemorrhage[1,2]. Thus, new medications with less adverse reactions need to be explored.

Along with the research progress on the pathogenic mechanism involved in ITP in recent years, directional immune intervention has received increasing interest as a treatment method for ITP.

Rituximab, a mouse/human chimeric monoclonal antibody against CD20 that was applied initially to CD20-positive B-cell lymphoma with success, is now also used to treat refractory ITP.

When combined with CD20 on B-lymphocytes, rituximab induces antibody/complement-dependent cytotoxicity and inhibits B-lymphocytes from producing autoantibodies and activating autoreactive T cells through its function as an antigen-presenting cell.

B-lymphocytes coated with rituximab may also compete with sensitized platelets to combine with Fc R, resulting in decreased platelet destruction[3].

Multi-site clinical trials witnessed good efficacy in rituximab either alone or in combination with dexamethasone in treating ITP as a second-line regimen with prolonged maintenance of response in most subjects and without severe adverse reactions or severe infectious complications[1,4-9].

Given that rituximab needs four to six weeks to take effect, this medication is characterized by prolonged efficacy with slow onset of the therapeutic effect. Thus, ITP patients need to boost platelets rapidly in the beginning of the therapy to decrease the risk of hemorrhage.

The concurrence of invalid platelet production and relative insufficiency of endogenous thrombopoietin (TPO), found in most IPT patients by recent studies, may be one of the potential factors impeding platelet production[10,11].

Capable of improving the formation of marrow megakaryocyte colonies in chronic ITP patients, TPO promotes platelets rapidly and lowers the risk of severe hemorrhage in ITP patients. However, studies on the combination of these two medications remain few in the treatment of ITP worldwide.

This paper reports the successful treatment of 14 ITP patients from May 2009 to December 2012 with low-dose rituximab in combination with recombinant human thrombopoietin (rhTPO).

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Patients and Methods

General Information

From May 2009 to December 2012, 14 ITP patients were treated with low-dose rituximab in combination with rhTPO. Among these patients were 1 male and 13 females aged 18 to 76 years (median age=52 years), with a disease history from three months to 18 years who were diagnosed with ITP according to the criteria previously reported[1]. These patients had no response to previous treatment of glucocorticoids. One patient had a recurrence post-splenectomy and 10 patients had no response to or relapsed after previous treatment with immunoglobulins, vincristine, danazol, or cyclosporine. This study, conducted in accordance with the Declaration of Helsinki, was approved from the Ethics Committee of Qing-dao Central Hospital. Written informed consent was obtained from all participants.

Blood platelet count (BPC) prior to treatment was 1×10^9/L to 18×10^9/L. Hemorrhage of skin or mucous membrane in various degrees were observed in the patients, including two with hemorrhage of the digestive tract, three with hypermenorrhea, and one with subarachnoid hemorrhage. All patients were negative for hepatitis B, hepatitis C, and HIV.

Treatment

Intravenous infusion of 100 mg of rituximab (Mabthera, Roche, Basel, Switzerland) for 1 h to 2 h was performed on days 1, 8, 15, and 22 for 30 min after anti-hypersensitivity treatment by oral ingestion of 4 mg of chlorpheniramine maleate and intravenous administration of 40 mg of methylprednisolone.

The patients also underwent recombinant human thrombopoietin (rhTPO) 300 μg/kg/d ih for 14 d. The dosage was reduced and the frequency was lowered to twice a week if BPC > 50×10^9/L or discontinued if BPC > 100×10^9/L. Platelet concentrate was infused to patients with hemorrhage tendency and/or BPC < 10×10^9/L.

Blood Routine

Blood routine was determined once or twice a week during the first month of treatment, twice a month during the second and third months, once a month during the fourth and sixth months, and then once every three months. Blood routine was re-examined once a patient manifested bleeding tendency. Serum immunoglobulins and lymphocyte subgroups (with flow cytometry) were determined pre- and post-treatment.

Criteria for Efficacy Evaluation

Complete response (CR)[12,13] was defined as BPC ≥ 100×10^9/L post-treatment and the absence of hemorrhage.

Response (R) was defined as BPC ≥ 30×10^9/L post-treatment with at least two occurrences of increase compared with BPC at baseline and the absence of hemorrhage.

No response (NR) was defined as BPC < 30×10^9/L post-treatment or less than two occurrences of platelet increase compared with the baseline or presence of bleeding.

At least two evaluations of BPC with an interval of more than 7 d were needed to confirm CR and R. The time of response was defined as the duration from the first dosage to the second occurrence of the first two successive laboratory results of BPC ≥ 30×10^9/L.

Relapse was considered as either of the following two cases: (1) BPC < 100×10^9/L post-CR or manifestation of hemorrhage OR (2) BPC < 30×10^9/L post-R or less than two occurrences of increase compared with baseline or manifestation of hemorrhage.

All evaluations were performed after hormonal treatment or other treatments were discontinued.

Statistical Analysis

Paired *t*-test was used to compare pre- and post-treatment data. $p < 0.05$ was considered statistically significant.

Results

Evaluation of Responses

Seven CRs (50%) and six Rs (43%) out of 14 adult ITP patients were observed, with an OR of 93%. A relapse was observed in one patient at the sixth month after CR was achieved. This patient underwent rituximab (100 mg qw) for another four times before CR was achieved, which was persistent for 18 months. Another relapse occurred in a patient at month 16 after CR was achieved. This patient was treated again with rituximab and achieved R after treatment for five months.

The median response time was 12 d (1 to 28). Three out of seven CR patients achieved R first and then CR after three to five months. Three out

of six Rs achieved CR but were converted to R. One NR (7%) patient with persistently lower BPC ($< 30 \times 10^9$/L) died from complications of pulmonary aspergillosis infection and cerebral hemorrhage after one month. One CR patient, who remained normal in BPC at D68, manifested interstitial pneumonitis and respiratory failure, and then died. The median follow-up was 17 (3 to 44) months (Table I).

Variation in Lymphocyte Subsets

B-lymphocytes with CD19 + CD20 + were eliminated after the treatment, whereas other lymphocyte subsets with CD3+, CD3 + CD4 +, CD3 + CD8 +, or CD3-CD56 + cells remained unchanged pre- and post-treatment (no statistical difference, Table II).

Serum Immunoglobulins

No variation was observed in serum immunoglobulins pre- and post-treatment (no statistical difference, Table III).

Adverse Reactions

One patient experienced fever with chills during the infusion of rituximab, but this patient recovered soon after the infusion was slowed down. Two patients complained of fatigue post-infusion. One 76-year-old diabetic patient manifested herpes zoster at day 20, which ameliorated after anti-virus treatment, but the medication was cancelled at day 22.

CR was achieved in one 63-year-old diabetic patient at day 33 post-treatment, but this patient died from interstitial pneumonitis at day 68 and was unresponsive to anti-viral and anti-inflammatory treatments, methylprednisolone, immunoglobulin, and adjuvant respiration with a respirator. Transient abnormality in coagulation was observed in two patients. This abnormality was mainly manifested as prolonged activated partial thromboplastin time (APTT) for more than 10 s compared with the normal level, and fibrinogen (Fg) decreased to below 1.5 g/L without obvious hemorrhage tendency. One of these patients was transfused with 400 mL of cryofresh plasma, whereas the other remained untreated, and both recovered soon.

Discussion

ITP is an acquired organ-specific autoimmune disease with the pathogenic mechanism of excessive destruction and inhibited platelet production. The inhibitory effect is caused by anti-platelet autoantibody generation of malfunctioning B-cells induced by disturbed regulation of T-lymphocytes *in vivo*. The preliminary results of multi-site clinical studies on rituximab in adult ITP patients showed a response rate (RR) of up to 55% to 57% without severe adverse reactions or severe infectious complications[7-9]. Out of 14 patients, we reported CR of 50% and R of 43% with OR of 93%, which is superior to the present results available from studies on rituximab used alone, which might be correlated to the combination with rhTPO.

No final conclusion was formed on the opportune time when rituximab treatment should begin for the patient. Some reports dispute that its earlier application may promote sustained RR and re-

Table I. . PLT variation in 14 ITP patients prior and post to treatment.

Case	Before treatment	1w	2w	3w	4w	8w	12w	16w	20w	24w	Until now	Curative effect	Duration time
1	6	39	87	114	201	132	168	129	86	47	126	CR	43 months
2	11	17	25	38	44	42	41	67	54	71	63	R	28 months
3	12	15	19	27	23							NR	Died
4	3	71	88	90	73	116	174	152	119	97	85	R	21 months
5	9	22	31	43	27	54	61	68	71	109	131	CR	20 months
6	16	35	71	89	172	165	201	188	152	181	150	CR	19 months
7	4	37	56	53	89	110	134	125	117	131	79	R	18 months
8	5	17	19	38	35	50	67	75	77	80	105	CR	17 months
9	17	29	33	45	68	129	137	115	184	157	131	CR	11 months
10	8	16	31	29	35	47	50	57	61	67	59	R	8 months
11	20	29	36	35	51	67	70	68	77	80	80	R	7 months
12	9	16	23	25	87	132						CR	Died
13	1	67	135	98	112	97	89	90	81		81	R	5 months
14	12	21	74	190	246	164	137				137	CR	3 months

Table II. Variation of lymphocyte subsets of ITP patients prior and post to treatment (%).

Group	Case	CD3+	CD3+CD4+	CD3+CD8+	CD3-CD56+	CD19+CD20+
Before treatment	14	68.3 ± 5.6	29.3 ± 4.2	32.8 ± 6.5	7.7 ± 4.3	22.7 ± 7.5
After treatment	14	77.8 ± 7.9	38.6 ± 5.7	28.4 ± 7.1	5.3 ± 5.2	0.17 ± 0.3
p value		> 0.05	> 0.05	> 0.05	> 0.05	< 0.01

lapse-free rate. Two patterns[14,15] have been reported for the promotion of platelets in patients. The first pattern is the rapid response pattern, which occurs during the early stage of treatment with gradual promotion of platelets following one or two times of medication up to the peak at weeks 6 to 10. This pattern is observed clinically in the majority of patients. The second, featured as the promotion starting at weeks 6 to 8 post-treatment with a platelet peak that is rapidly achievable, is the delayed response pattern. However, an urgent need exists for IPT patients to combine medications capable of promoting platelets rapidly before rituximab takes effect.

Recent studies have found the concurrence of ineffective platelet production and insufficiency of endogenous TPO in most ITP patients, and this concurrence may be one of the potential factors impeding platelet production[10,11]. Capable of improving marrow megakaryocytes to form colonies in chronic ITP patients, TPO significantly increases platelets in peripheral blood, thus, lowering the risk of severe hemorrhage in ITP patients.

rhTPO produced by Yofoto Company (Ningbo, China) is a full-length glycosylated TPO expressed by ovarian cells of Chinese hamsters with definite capability to promote platelets[16,17].

The onset of therapeutic effects in the 13 response patients reported in this study was at day 12 (1 to 28), which is earlier than that in patients treated with rituximab alone in previous reports[7-9]. However, rhTPO alone cannot sustain the long-term efficacy in promoting platelets, and an abrupt drop of platelets may occur once the medication is discontinued. Thus, the problem of platelet reduction following the suspension of rhTPO may also be solved when rhTPO is combined with rituximab.

Previous studies concluded that rituximab eliminates CD19 + CD20 + B-lymphocytes but does not influence numbers of T cells or NK cells, without significant variations in serum IgG, IgM, and IgA pre- and post-treatment, which is consistent with the results observed in our research. To date, no clinical or laboratory parameters have been found reliable in most studies for predicting the therapeutic results of rituximab, which seem unaffected by factors including platelet count, therapeutic approach, sex, age, serum immunoglobulins level, quantity of B-lymphocytes, antibodies against platelets, and blood concentration of rituximab. However, this result should be confirmed by controlled clinical trials with larger samples treated for prolonged periods.

Previous studies have reported the transient effect of rituximab to eliminate B-lymphocytes in circulation, which may recover gradually after the medication is discontinued. These findings may be correlated to the recurrence observed in some patients. In this research, recurrence was observed in two patients who showed response to re-application of rituximab. Hasan et al[18] observed the efficacy of re-application of rituximab with a standardized dosage in 20 recurrent patients who were responsive to the initial identical treatment, with similarity in RR, onset of therapeutic effect, and good compliance between the two applications, suggesting the feasibility of reuse of rituximab for relapse following the same medication.

The main adverse reactions, as suggested by

Table III. Variation of serum immune globulin in ITP patients prior and post to treatment (g/L).

Group	Case	IgG	IgM	IgA
Before treatment	14	13.52 ± 5.61	1.33 ± 0.86	1.66 ± 0.72
After treatment	14	11.35 ± 6.24	1.04 ± 0.75	1.49 ± 0.85
p value		> 0.05	> 0.05	> 0.05

previous research[19], include cytokine-release syndrome, which does not generally influence the treatment and is patent in the first infusion. Similarly, fever with chills was observed in one patient infused with rituximab intravenously, who recovered immediately after the infusion was slowed down. Two patients experienced fatigue after infusion, and these patients recovered in hours. However, transient abnormality of coagulation in two patients with unclear causality needs further observation to clarify its potential correlation to rituximab or rhTPO.

In this research, severe pulmonary complications caused death in two senile patients with concurrent diabetes. Pulmonary injuries induced by rituximab have been reported by several studies worldwide[18-21], which remain more frequent in the elderly and in children, suggesting the need to weigh cautiously the advantages and the disadvantages of rituximab for the senile.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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