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Efficacy of standard dose rituximab for refractory idiopathic thrombocytopenic purpura in children

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Abstract. – OBJECTIVE: The present study intends to investigate the efficacy and safety of standard dose rituximab for treatment of refractory idiopathic thrombocytopenic purpura (RITP) in children.

PATIENTS AND METHODS: A total of 50 cases of children hospitalized with RITP in a hospital were enrolled in this study, and randomly divided into two treatment groups according to the therapeutic methods: rituximab group (n = 26) and vincristine group (n = 24). Another 20 healthy children receiving physical examination in the hospital during the corresponding period were enrolled as the control group. Before treatment the thrombocytes were counted with hematology analyzer and the $CD19^+/CD20^+$ B cells in peripheral blood tested with flow cytometry in rituximab group. Then the children in the rituximab group were given standard dose rituximab at 375 mg/m^2 via four weekly intravenous drip, while those in vincristine group treated with vincristine at 0.02 mg/kg by intravenous drip once a week for three months. During the treatment the adverse drug reactions were observed and recorded. After the treatment, the efficacy of two drugs was each evaluated, and the thrombocytes and $CD19^+/CD20^+$ B cells in peripheral blood of rituximab group were quantified in the same way, and the children in both treatment groups were followed up and the recurrence rate recorded.

RESULTS: The total efficiency including complete response and partial response in rituximab group was significantly higher than that in vincristine group (69.2% vs. 37.5%, $\chi^2 = 9.74$, $p < 0.01$). The prevalence rates of adverse reactions were statistically indifferent between two treatment groups during the therapy (11.5% vs. 8.3%, $\chi^2 = 0.62$, $p > 0.05$). The follow-up visit showed that the recurrence rate of rituximab group including those showing complete response and partial response was significantly lower than that of vincristine group (22.2% vs. 55.6%, $\chi^2 = 7.24$, $p < 0.05$). The peripheral blood platelet number of children showing complete response and partial response in group of rituximab was 106.7 ± 32.5 × 10^9/L after treatment and significantly higher compared with that before treatment ($t = 12.48$, $p < 0.01$). The amount of $CD19^+/CD20^+$ B cells in peripheral blood of rituximab group after treatment was significantly lower than that before treatment ($t = 6.71$, $p > 0.05$).

CONCLUSIONS: Rituximab may play a role in the efficacy by depleting B cells and can cure RITP in children without causing serious adverse reactions.

Key Words:
Idiopathic thrombocytopenic purpura, Children, Rituximab, Standard dose, Efficacy.

Introduction

Idiopathic thrombocytopenic purpura (ITP) is a common hemorrhagic disease in childhood with excessive platelet destruction caused by autoimmune disorder. Although about 70% of the paediatric patients of ITP may achieve spontaneous improvement within 6 months[1] or about 30.4% (17/56) children with chronic ITP achieved the spontaneous improvement at an average age of 8.5 years[2]. However, 42.9% of pediatric patients (24/56) turned into intractable problem in clinic[2] or chronic refractory ITP (RITP) with repeated attacks. The standard clinical therapy for children with chronic RITP is corticosteroid therapy, intravenous immunoglobulins, anti-D immune globulins, or splenectomy. The treatment options are limited in the clinic to.

And more drugs are introduced for new immune-adjustment treatment solution for ITP patients. However, there is still lack of unified and more effective therapy of ITP, especially for those below 3 years old and unable to response to glu-

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cocorticoid or n favor of other treatment instead of splenectomy. Hopefully, a human-mouse chimeric anti-CD20 monoclonal antibody, known as rituximab, was initially applied in treatment of recurrent low-grade malignant lymphoma and is now used for treatment of autoimmune diseases including RITP, forming an effective and safe therapeutic approach for ITP including corticosteroid-resistant ITP[3] by obviating or delaying the need for a splenectomy and demonstrating some efficacy[4-5] in more than half of all adults and children[1,6-9]. However, there are still lacks of closely related randomly controlled trials of the clinical safety and effectiveness of rituximab in treatment of children with RITP in China.

Patients and Methods

Clinical Data

Totally 50 children with RITP, 16 males and 34 females, at 9 months to 13 years of age (with an average age of 5.6 ± 2.1 years), with a platelet count at a baseline of 4.0-30.0 × 10^9/L (averaging at 18 × 10^9/L)[10], hospitalized between June 2007 and April 2011 were considered refractory[11] and enrolled in this study since hormone or large dose gamma globulins therapy was previously ineffective or poorly effective for all the patients. The duration of disease was from 11 months to 9 years before treatment, with an average of 2.3 ± 0.8 years. They were divided into two groups according to different therapeutic methods: rituximab group (n = 26) and vincristine group (n = 24). The distribution differences in age, sex, course of disease, and peripheral blood platelet count on admission were comparable and statistically indifferent between two treatment groups ($p > 0.05$). Another 20 cases of healthy children who, without tumor and autoimmune diseases, received physical examination in the hospital during the corresponding period, were enrolled as the healthy control group. And the differences in age and sex distributions between the healthy control group and each of the treatment groups were statistically indifferent (each at $p > 0.05$).

Therapeutic Method

The children in the rituximab group were intravenously injected with 375 mg/m^2 of standard dose rituximab (Mabthera, made by Roche, Basel, Switzerland) weekly for 4 weeks. The immunosuppressive agents, chemotherapy drugs, anticoagulants, hormonal shock therapy were not used during the treatment. The children in the vincristine group were intravenously injected with 0.02 mg/kg of vincristine (Shanghai Hualian Pharmaceutical Co., Ltd.) for 8 hours weekly for 3 months. The children in two groups were simultaneously given with other symptomatic and supportive therapies.

Outcome Measures

1. **Efficacy and adverse drug reactions.** The efficacies of the treated children with drugs were evaluated after treatment. The adverse drug reactions were observed and recorded during the treatment. The efficacy was assessed according to the routine rules[9,11]: a. complete response: platelet count is over 150 × 10^9/L; bleeding symptom is stopped; b. partial response: platelet count is (50-150) × 10^9/L; bleeding symptom is stopped; c. minimal response: platelet count is elevated; bleeding symptom is improved; d. no response: platelet count (< 30 × 10^9/L) and bleeding symptom are not improved. And the response rate covers complete response and partial response.
2. **Recurrence rate during follow-up visits.** The children in both rituximab and vincristine groups were followed up and the recurrence rate was collected in 6-24 months after treatment. The recurrence was defined as the recovery of one or more of the following cases: a. those achieved complete response, with platelet count below 100 × 10^9/L or bleeding symptom; b. those achieved partial response or minimal response, with platelet count of below 50 × 10^9/L or platelet count increase below 2 folds of baseline or bleeding symptom.
3. **Effects of rituximab on the number of $CD19^+/CD20^+$ B cells in peripheral blood.** Three milliliters of fasting venous blood were respectively sampled from children each in the rituximab group before and after treatment, while three milliliters of fasting venous blood were drawn from children in the healthy control group during physical examination. The presence of B cell-related surface markers including CD19 and CD20 in the blood sample was individually detected using the flow cytometer (Beckman, Brea, CA, USA). Cellquest software was used for analysis of the results.

Statistical Treatment

A database was established using SPSS 12.0 statistical software (SPSS Inc., Chicago, IL, USA). A two-sample *t*-test was used for inter-

Table I. Comparison of efficacy between treatment groups [n(%)]

Treatments	n	Complete response	Partial response	Minimal response	no response
rituximab group	26	11(42.3)	7(26.9)	3(11.5)	5(19.2)
vincristine group	24	3(12.5)	6(25.0)	4(16.7)	11(45.8)

group comparison of measured data. A chi-square test was used for comparison of enumeration data. The value of $p < 0.05$ was defined as statistical difference.

Results

Efficacy and Adverse Drug Effects in Treatment Groups

The response rate of rituximab group was significantly higher than that of vincristine group (69.2% *vs.* 37.5%, $\chi^2 = 9.74$, $p < 0.01$). The efficacies were shown in Table I.

During the treatment, there were 3 cases of children with adverse drug reactions in the rituximab group, including one case with mild rash and 2 cases with abnormal liver and kidney function, and 2 cases of children with abnormal liver and kidney function in the vincristine group. The difference in the prevalence of adverse reactions between two groups was not statistically significant (11.5% *vs.* 8.3%, $\chi^2 = 0.62$, $p > 0.05$). And no infection was found both in rituximab group and in vincristine group.

Comparison of Follow-up Results in Two Groups

During the follow-up visits of 6 to 24 months four (22.2%, 4/18) children with RITP, including complete response and partial response, were found with recurrence in the rituximab group, while five (55.6%, 5/9) children with FITP, including complete response and partial response, with recurrence in the vincristine group; the recurrence rates were significantly different between two treatment groups ($\chi^2 = 7.24$, $p < 0.05$).

Effects of Rituximab on Platelet Count and $CD19^+/CD20^+$ B Cells in Children with RITP

The children in the rituximab group were further divided into the effective group (complete response and partial response) and ineffective group (minimal response and no response) according to the efficacy. The peripheral blood platelet count of children in the effective group after rituximab treatment was significantly increased to $(106.7 \pm 32.5) \times 10^9$/L compared with that before treatment ($t = 12.48$, $p < 0.01$). The difference in peripheral blood platelet count of children in the ineffective group before and after treatment was not statistically significant ($t = 0.85$, $p > 0.05$). The number of $CD19^+/CD20^+$ B cells in peripheral blood of children in both effective and ineffective groups before treatment were significantly higher than that in the healthy control group ($t = 9.85$ & 7.61, both at $p < 0.01$). The number of $CD19^+/CD20^+$ B cells in peripheral blood of children in the effective group after treatment was significantly lower than that before treatment ($t = 6.71$, $p < 0.05$). The difference in the number of $CD19^+/CD20^+$ B cells in peripheral blood of children in the ineffective group before and after treatment was statistically indifferent ($t = 0.38$, $p > 0.05$). The results were shown in Table II.

Discussion

In recent years, the pathogenesis of ITP has been considered to be closely related to the disorder of humoral immunity and accompanied by a significant increase in B cells which have anti-apoptotic activity and continuously produce a large amount of anti-platelet autoantibodies. The excessive antibodies lead to autoimmune disorder of thrombocytopenia and promote the occurrence and development of ITP. As a chimeric IgG antibody, rituximab directly inhibits B cell growth or induce B cell apoptosis. Rituximab appears safe and well-tolerated in children[9], and 26 children with 11 months to 9 years of RITP before treatment in this study, including children with complete response and partial response, achieved significantly higher response rates in standard dose rituximab group than in vincristine group (69.2% *vs.* 37.5%, $p < 0.01$), indicating rituximab instead of vincristine is a optimal therapeutic regimen for children with RITP. According to a study in a small number of cases with the rituximab doses from 50 to 375 mg/m^2, lower

Table II. Quantity of peripheral platelet and CD19+/CD20+ B cells before and after treatment in rituximab group.

	n	platelet count (109/L)	CD19+/CD20+ B cells (%)
Effective			
before treatment	18	21.5 ± 9.8	27.5 ± 4.7
after treatment	18	106.7 ± 32.5	12.4 ± 2.8
Ineffective			
before treatment	8	22.6 ± 9.4	27.2 ± 5.1
after treatment	8	35.9 ± 11.9	25.8 ± 4.2
Healthy control	20	3(12.5)	11.6 ± 2.4

doses were less likely to reach necessary response rate than higher doses. Further, the treatments with four once-weekly doses of 100 mg and a single dose of at 375 mg/m^2 of rituximab in 22 pediatric RITP patients achieved 45% and 59% of respond rate respectively in ITP chileren[1]. In some studies the response with lower dose rituximab (100 mg/m^2) is slower than with standard dose (375 mg/m^2) both at a basis of four weekly administrations[12]. Also, the number of peripheral blood platelet in this study was markedly increased from initial average of 18 × 10^9/L to 106.7 ± 32.5 × 10^9/L after therapy with standard dose rituximab (t = 12.48, $p < 0.01$). A much similar increase trend of platelet count was found in a rituximab treatment at the same dose for children with RITP in complete response (15/24 cases) and partial response (2/24 cases)[9]. In this study the treatment with rituximab at standard dose of 375 mg/m^2 realized a higher response rate of 69.2%, indicating the standard dose of rituximab is a preferred choice for pediatric RITP cases. One of the strategies related to doses in adults with RITP is the combination of low-dose rituximab (100 mg/m^2) and high-dose dexamethasone (40 mg/d for four days) as frontline therapy which achieved an overall response of 90.5% at day 28[13]. Similar strategies need to be studied in children with RITP. Other researches indicated the response rates of 32% (27 cases) and 63% (8 cases) in rituximab respectively at a lower and the standard dose in children at 2.0-19.0 years of age, with response duration of 9-104 weeks[1]. The response rates of 69.2% in rituximab therapy of this study is also higher than the above mentioned 63% and 32%, implying that a higher dose or possibly the sample sizes or at least the both may affect the response rates in clinical experiments. Therefore, the response duration and the response rates of rituximab by children with RITP need to be further studied with a larger sample sizes in a prolonged observation period, because the delayed responses may occur both in the lower dose and in standard dose regimens[12].

The difference in the occurrence of adverse reactions between two groups was statistically indifferent during treatment (11.5% *vs.* 8.3%, $p > 0.05$). It was demonstrated that rituximab therapy of children with RITP had a definite efficacy without side effects and no novel or substantial long-term clinical toxicity was observed 5 years after administration[11]. And the recurrence rate of rituximab group including complete response and partial response was significantly lower than that of vincristine group during follow-up visit (22.2% *vs.* 55.6%, $p < 0.05$). Meanwhile, no infection was found either in two treatment groups of this study or in other rituximab therapy at standard dose where immunoglobulin levels of the children cases decreased to below the normal range[9]. Therefore, the efficacy is coupled with the safety in the therapy of pediatric RITP with standard dose rituximab.

It is traditionally considered that rituximab killed B cells primarily by inducing B cell apoptosis or by mediating complement and antibody-dependent cytotoxicity pathway. The pathway was coordinated by complement's Cq and Fc receptors of a variety of effector cells including macrophages, T cells, and natural killer cells[14,15]. Recent research suggested that standard dose rituximab performed as an immunomodulating adjunct for treatment of RITP by eliminating B-lymphocytes producing inhibitory ADAMTS-13 autoantibodies[9,11]. According to recent hypothesis[16] the rituximab-opsonized B cells will be recognized by monocytes and macrophages; these effector cells would be diverted away from interactions with autoimmune antibody complexes. The number of CD19$^+$/CD20$^+$ B cells in peripheral blood of effective children and the number of ineffective children with RITP before treatment with rituximab were each significantly higher than that of the healthy control group (t = 9.85 and 7.61, each at $p < 0.01$) in this study, demonstrating an abnormal expression CD19$^+$/CD20$^+$ B

cells and increased B cell activity in pediatric RITP cases. The number of $CD19^+/CD20^+$ B cells in the effective group was significantly decreased after rituximab treatment, while the difference before and after treatment was significantly indifferent among the ineffective subjects. Administration of rituximab at standard dose may play an indirect therapeutic role in children with RITP by short-term and long-term B-lymphocyte depletion in children with RITP[17]. Although the serum sickness may happen more in children than in adults during therapy with rituximab, the appearance of possible infection due to B-lymphocyte depletion did not occur unless in those with an underlying predisposition to infections[17]. However, the optimal regimen and related factors affecting the efficacy are to be further explored.

Conclusions

Standard dose rituximab at 375 mg/m^2 is an effective and preferred therapy for children with RITP, without serious adverse reactions. It also reduces the recurrence rate in children and becomes a new choice in the front-line treatment of these children.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Bernadette G. Rituximab in the treatment of autoimmune haematological disorders. Br J Haematol 2008; 141:149-169.
2) Motohiro K, Katsuyoshi K, Akira K, Ryoji H. Spontaneous improvement of chronic immune thrombocytopenia in children: experience of 56 patients at a single institute. Int J Hematol 2012; 96: 729-732.
3) Zhang C, Liu HF, Chen XH, Gao L, Gao L, Liu Y, Kong PY, Sun AH, Zhang X. Is Splenectomy necessary for immune thrombocytopenic purpura? The role of rituximab in patients with corticosteroid resistance in a single-center experience. Clin Ther 2014; 36: 385-388.
4) Wang J, Wiley JM, Luddy R, Greenberg J, Feuerstein MA, Bussel JB. Chronic immune thrombocytopenic purpura in children: assessment of rituximab treatment. J Pediatr 2005; 146: 217-221.
5) Taube T, Sehmid H, Reinhard H, von Stackelberg A, Overberg US. Effect of a single dose of rituximab in chronic immune thrombocytopenic purpura in childhood. Haematologiea 2005; 90: 281-283.
6) Bay A, Oner AF, Uner A, Dogan M. Use of rituximab in chronic childhood immune thrombocytopenic purpura. Pediatr Int 2006; 48: 514-516.
7) Bennett CM, Rogers ZR, Kinnamon DD, Bussel JB, Mahoney DH, Abshire TC, Sawaf H, Moore TB, Loh ML, Glader BE, McCarthy MC, Mueller BU, Olson TA, Lorenzana AN, Mentzer WC, Buchanan GR, Feldman HA, Neufeld EJ. Prospective phase 1/2 study of rituximab in childhood and adolescent chronic immune thrombocytopenic purpura. Blood 2006; 107: 2639-2642.
8) Moschovi M, Trimis G, Pergantou H, Platokouki H, Vrachnou E, Tzortzatou-Stathopoulou F. Clinical remission following monoclonal anti-CD20 therapy in two children with chronic refractory idiopathic thrombocytopenic purpura. J Paediatr Child Health 2005; 41: 384-386.
9) Julie W, Joseph MW, Ruth L, Jay G, Michael AF, James BB. Chronic immune thrombocytopenic purpura in children: assessment of rituximab treatment. J Pediatr 2005; 146: 217-221.
10) Bennett CM, Rogers ZR, Kinnamon DD, Bussel JB, Mahoney DH, Abshire TC, Sawaf H, Moore TB, Loh ML, Glader BE, McCarthy MC, Mueller BU, Olson TA, Lorenzana AN, Mentzer WC, Buchanan GR, Feldman HA, Neufeld EJ. Prospective phase 1/2 study of rituximab in childhood and adolescent chronic immune thrombocytopenic purpura. Blood 2006; 107: 2639-2642.
11) Patel VL, Mahévas M, Lee SY, Stasi R, Cunningham-Rundles S, Godeau B, Kanter J, Neufeld E, Taube T, Ramenghi U, Shenoy S, Ward MJ, Mihatov N, Patel VL, Bierling P, Lesser M, Cooper N, Bussel JB. Outcomes 5 years after response to rituximab therapy in children and adults with immune thrombocytopenia. Blood 2012; 119: 5989-5995.
12) Kelly K, Gleeson M, Murphy PT. Slow responses to standard dose rituximab in immune thrombocytopenic purpura. Haematologica 2009; 94: 443-444.
13) Gómez-Almaguer D, Tarín-Arzaga L, Moreno-Jaime B, Jaime-Pérez JC, Ceballos-López AA, Ruiz-Argüelles GJ, Ruiz-Delgado GJ, Cantú-Rodríguez OG, Gutiérrez-Aguirre CH, Sánchez-Cárdenas M. High response rate to low-dose rituximab plus high-dose dexamethasone as frontline therapy in adult patients with primary immune thrombocytopenia. Eur J Haematol 2013; 90: 494-500.
14) Friedber JW. Unique toxieities and resistance mechanisms associated with monoelonal antibody therapy. Hematology Am Soc Hematol Educ Program 2005; 329-334.
15) Smith MR. Rituximab (Monoelonal anti-CD20 antibody): mechanisms of action and resistance. Oncogene 2003; 22: 7359-7368.
16) Montoya RC, Poiesz BJ. Rituximab as prophylaxis in chronic relapsing thrombotic thrombocytopenic purpura: a case report and review of the literature. Blood Coagul Fibrinolysis 2012; 23: 338-41.
17) Cooper N, Bussel JB. The long-term impact of rituximab for childhood immune thrombocytopenia. Curr Rheumatol Rep 2010; 12: 94-100.