Lower dose rituximab is active in adults patients with idiopathic thrombocytopenic purpura

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ABSTRACT

Rituximab 375 mg/m^2 weekly for four weeks has significant activity in patients with immune thrombocytopenia. We evaluated the activity of lower dose rituximab (100 mg iv weekly for 4 weeks) in 28 adults with idiopathic thrombocytopenic purpura. Overall (platelet count > 50×10^9/L) and complete responses (platelet count > 100×10^9/L) were achieved in 21/28 (75%) and 12/28 (43%) patients respectively. The median time to response and time to complete response were 31 and 44 days respectively. After a median follow-up of 11 months (range 3-18), 7/21 (33%) patients relapsed and 3 needed further treatments. In patients with idiopathic thrombocytopenic purpura, lower dose rituximab seems to show similar activity to standard dose.

Key words: immune thrombocytopenia, B-cell depletion, lower dose rituximab.

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Introduction

Rituximab is an emerging new agent for the treatment of several autoimmune disorders[1-9] and, in particular, many reports highlighted the effect of rituximab in idiopathic thrombocytopenic purpura (ITP).[10-18] To date, rituximab has been administered with the same schedule proposed for B-cell lymphomas, i.e. 375 mg/m^2 every seven days for four weeks. Very few dose-finding and pharmacokinetic studies have been performed in autoimmune diseases.[19] In this context, the B-cell total mass is much less than in patients with lymphoma and, therefore, a reduced dosage of rituximab might still be sufficient for its therapeutic purpose. Based on this assumption, we performed a prospective clinical trial using lower dose rituximab in patients with ITP. We explored response rates of lower dose rituximab and correlated results with biological findings.

Design and Methods

Patients

From February 2006 to April 2007, 28 consecutive adult patients (median age 43 years, range 16-71 years) with ITP that had relapsed or were refractory at least to a full course of steroid therapy were included in this prospective, multicenter, phase II study, approved by the ethical boards of the co-ordinating center. Patients' inclusion criteria were: a diagnosis of ITP according to the guidelines of the American Society of Hematology,[20] active symptomatic disease or in need of chronic treatment with steroids to maintain a safe number platelet count (≥20×10^9/L), previous treatment with at least one line of therapy, written informed consent. Exclusion criteria were: HIV, HCV serology or HBsAg positivity, positive pregnancy test, malignant diseases. A previous effective treatment with standard dose rituximab was not considered an exclusion criterion.

Patients' main characteristics are summarized in Table 1. The relatively high median platelet count at baseline (31×10^9/L, range 6-114×10^9/L) reflected the concomitant administration of steroids in a large number of cases (21/28 patients) when rituximab was started. Therefore, all patients included in this study were representative of a population of symptomatic ITP requiring treatment.

Treatment

Rituximab was given at the fixed dose of 100 mg administered as an intravenous (iv) infusion weekly (on day 1 of weeks 1, 2, 3 and 4). The choice to use this fixed dose was rather empirical and also based on administration convenience. Patients received oral acetaminophen 500 mg and iv chlorphenamine 10 mg as pre-medication therapy. No other

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cytotoxic or immunosuppressive drugs were given in association with rituximab. Patients with steroid dependency to maintain a low platelet count >20×10⁹/L or active bleeding before rituximab administration were allowed to continue steroids also during rituximab therapy at the individual minimal effective dosage sufficient to maintain a safe number of platelets. Nevertheless only patients that reached steroid discontinuation during or soon after rituximab were considered for response.

Response criteria

The evaluation of the response was made monitoring the platelet count every week during the first month of treatment, then at bi-weekly intervals up to the sixth month and then monthly. A complete response (CR) was defined as a platelet count >100×10⁹/L and discontinuation of the steroid therapy. This level of platelets was chosen in accordance with our previous reports.[9,14,16] However, we also evaluated the rate of patients who achieved a complete normalization of platelet count (i.e. >150×10⁹/L). Partial response (PR) was defined as a platelet level between 50 and 100×10⁹/L and discontinuation of the steroid therapy. An overall response (OR) was defined as a partial or complete response. Patients with less than PR were considered non-responders (NR). Patients who required steroid administration during rituximab therapy were considered responders only if a steroid discontinuation (previously not possible) was achieved. The hematologic improvement was also assessed evaluating the time to response (TTR; i.e. the time necessary to reach a platelet count ≥50×10⁹/L) and the time to complete response (TCR; i.e. the time necessary to reach CR). For relapse free survival (RFS) we considered the whole period of spontaneous maintenance of the best response achieved (i.e. CR or PR). For treatment free survival (TFS) we considered the period between response to rituximab and the need for further therapy because of symptomatic or severe (<20×10⁹/L) thrombocytopenia. Therefore, those patients who, during the period of observation after rituximab, lost their best response but did not require further treatment were not considered as failure. Rituximab-related toxicity was assessed during the period of treatment and during the follow-up. Clinical and laboratory side effects were evaluated and graded according to the WHO scale.

B-cell depletion assessment

In order to evaluate B-cell depletion after rituximab, peripheral blood immunophenotypic analyses of CD19-CD20 lymphoid markers were performed in 21 available patients at baseline and then monthly from the beginning of treatment.

Blood sampling and pharmacokinetic analysis

Blood sampling for rituximab determination was performed at weeks +4, +8, +10 and +12 after the start of the therapy in a total of 8 patients. Samples from all time points were stored at –20°C until analysis. Rituximab concentration-time data were analyzed using a statistical pharmacokinetic population software (P-Pharm, version 3, Simed, Creteil, France). In all cases, rituximab plasma concentration was determined using a previously validated enzyme-linked immunoassay (ELISA.[21]

Table 1. Main clinical and laboratory features of patients before rituximab treatment.

Patients	28
Median age, years (range)	43 (16-71)
Males/females	19/9
Median weight, kg (range)	72 (42-112)
Median surface area, m² (range)	2 (1.4-2.3)
Diagnosis-rituximab median interval, months (range)	26 (1-451)
Median platelet count before rituximab, x10⁹/L (range)	31 (6-114)
Previous treatments	
Steroids	28
High dose IVIG	9
Azathioprine	3
Danazole	2
Standard dose rituximab	2
Vincristine and cyclophosphamide	1
Splenectomy	1

Statistical analysis

Stepwise logistic regression was used to assess whether response to low dose rituximab in patients with ITP was associated with age, gender, diagnosis-rituximab interval and baseline CD20⁺ lymphocytes. Results were considered statistically significant when $p \leq 0.05$. Graphs of RFS and TFS were achieved using the Kaplan-Meier method. Statistical analyses were performed in Stata/SE 9.0 for Windows.

Results and Discussion

All 28 patients included in the study completed the therapeutic program receiving the four infusions of rituximab as scheduled. Overall, CR and PR rates were 21/28 (75%), 12/28 (43%), 9/28 (32%) respectively (Table 2). Ten out of 28 patients (35%) achieved a complete normalization of platelet count (i.e. > 150×10⁹/L). The 2 patients previously treated with standard dose rituximab again achieved the same type of response: 1 CR still lasting after nine months of follow-up and 1 PR lasted four months but with up till now no need of further treatments (17 months follow-up). The TTR to rituximab standard and low dose rituximab of these 2 patients was similar (7 and 60 days). Stepwise logistic regression showed that both CR and OR were associated with age. CR showed an odds ratio=0.94, CI 95% [0.90; 0.99] and OR an odds ratio=0.93, CI 95% [0.86; 0.99], showing that as age increases the complete or overall response probability decreases. TTR was observed within day +7 in 5 out of 16 evaluable responding patients (31%) and in all remaining cases after day 22, with a median time of 31 days. Median TCR was 44 days (range 7-90). The median time of observation, in responding patients, was 11 months

(range 3-18). Seven out of 21 responding patients (33%) relapsed [3/12 CR after two (n=1), and three months (n=2) and 4/9 PR after three, (n=1) and four months (n=3)] relapsed and 3 needed further treatments. Eleven month cumulative RFS and TFS probabilities were 64% and 78% respectively (Figure 1). We did not observe any infusion-related reactions. None of the patients experienced opportunistic or other severe infections and there was no evidence of significant hematologic or non-hematologic toxicity. All patients achieved complete B-cell depletion, with a median B-lymphocyte count of 0.22×10^9/L (range 0.04-0.96) at baseline, 0.00×10^9/L (range 0.00-0.02) at month 2 and 0.02×10^9/L (range 0.00-0.07) at month 6. The median plasma level of rituximab just after the last infusion was 25.6 μg/mL (range: from 0 μg/mL to 77.9 μg/mL) (Table 2). Thereafter, rituximab concentrations decreased following a decay characterized by a median elimination half-life of 536 hours (range: 377.5-665 hrs.) and detectable levels were reported till week 12 after the start of therapy in all but one patient. The median plasma concentrations at 8, 10 and 12 weeks after the start of therapy were 13 μg/mL (range: 0-29 μg/mL), 5 μg/mL (range: 0-24 μg/mL) and 4 μg/mL (range: 0-12 μg/mL), respectively.

This study shows that with lower dose rituximab (approximately one seventh of the approved standard dose in lymphoma) the level of B-cell depletion and the response rates appear similar to those previously observed with standard dosages in a population of ITP patients with the same characteristics.[14,16] Younger age correlated with better response rate, while no relationship could be found between response rate, gender, diagnosis-rituximab interval and baseline $CD20^+$ lymphocytes. Our experience confirms the results of Provan and co-workers with the same 100 mg schedule adopted for this study. In 11 patients with various autoimmune pancytopenia the Authors reported activity in 4/7 patients with ITP, 1/1 with autoimmune pancytopenia, 1/1 with autoimmune hemolytic anemia, while no effect was observed in 1/1 patient with pure red cell aplasia and in 1/1 with autoimmune neutropenia.[22] Data concerning the timing of response were remarkably different to our previous experience[14,16] and with the results of other studies[12,13] with standard dose. It must be remarked, however, that some other groups did not highlight this prompt response in the majority of patients treated with standard dose.[15,17] The fact that we did not observe any infusion-related reaction appears probably related both to the baseline lower B-cell mass and to the lower dose of rituximab employed. Rituximab pharmacokinetic data showed a concentration time-course profile and a linear decay that was similar, once corrected for the difference in the dose, to that observed in previous studies with standard dose in patients with lymphoma and autoimmune disorders.[21] Our data show an elimination half-life of 535.6 hours (range: 377.5-664.7 hours), which is quite similar to that of 484.7 hours (range: 292.5-858.6 hours) previously found.[21] Rituximab plasma levels remained detectable for up to eight weeks following completion of treatment. Furthermore, this data suggest that anti-CD20 therapy for immune thrombocytopenia, as well as for other autoimmune diseases, should be scheduled differently to that for lymphoproliferative disorders, possibly also with dramatic cost improvement. Recently, Stasi and co-workers investigated the effect of B-depletion after rituximab to revert abnormalities of T-cell subsets in patients with ITP.[23] An increased pre-treatment Th1/Th2 and Tc1/Tc2 ratios together with an increased expression of Fas ligand and Bcl-2 mRNA and a decreased expression of Bax mRNA in Th cells, were inverted in responders after treatment, whereas they remained unchanged in non-responders. Our findings suggest that probably a similar biological effect may be pursued also with low dose rituximab. However, since all patients achieve B-cell depletion but only some of them respond to rituximab, it seems that other different pathogenic mechanisms are involved in the development and the maintenance of ITP. In the future, the identification before treatment of a patient's specific pathway might help optimize the therapy for this disease. In conclusion,

Table 2. Plasma concentrations of rituximab in groups with autoimmune disorders and ITP-low dose treated with weekly administrations. Data are reported as medians (range).

Sampling time	*Rituximab plasma concentration (mg/mL)*	
	Autoimmune disorders[21]	*ITP-low dose*
N. of patients	14	8
Rituximab dose	375 mg/m^2	100 mg
	Follow-up after the start of therapy (week)	
4	194 (55.5-261)	26 (0-78)
5	137 (38-172.5)	
6	86 (14-148)	
7	63 (28-88)	
8	41 (30-56)	13 (0-29)
9	25 (18-109)	
10		5 (0-24)
12		4 (0-12)

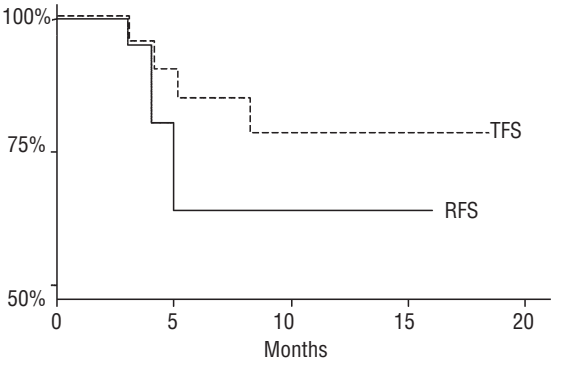

Figure 1. Relapse free survival (RFS —) and treatment free survival (TFS - - -) in 21 patients responding after rituximab treatment.

this study confirms the safety and the high potential activity of rituximab in patients with ITP. Furthermore, it highlights the importance for a more disease-specific therapeutic schedule and the need for randomized studies. Lower dose rituximab led to response rates similar to standard dose but with a slower timing of response.

Authorship and Disclosures

FZ, MB, FF, MB, RF: research conception and design, data analysis; FZ, MB, MR: wrote the paper; MR, MM, MAA: performed pharmacokinetic study; FZ, MTP, SP, LM, MC, MB, MD, MI, FP, NV: performed research, and analyzed and interpreted data; MI and FS: performed statistics. All the authors gave their approval of the final version. The authors reported no potential conflicts of interest.

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