The B-cell compartment as the selective target for the treatment of immune thrombocytopenias

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Background and Objectives. Rituximab is a chimeric anti-CD20 monoclonal antibody active against normal and malignant B cells. Treatment with rituximab is associated with the development of a severe (even if transient) B-cell depletion from peripheral blood and lymphatic tissues. These effects could be useful in autoimmune diseases in order to interfere with the production of pathologic antibodies.

Design and Methods. To investigate this, we treated 20 patients with rituximab 375 mg/m^2 i.v. every 7 days for 4 times. These 20 patients all had active and symptomatic autoimmune thrombocytopenia that had relapsed or was refractory to standard therapies (15 had idiopathic thrombocytopenic purpura, 1 idiopathic thrombocytopenia and neutropenia, 2 thrombocytopenia and concomitant undifferentiated connective tissue disease, and 2 had thrombocytopenia and concomitant B-cell lymphoprolipherative disorders). Only treatment with steroids, if strictly necessary to maintain a safe number of platelets, was allowed during the period of rituximab administration, but only patients who reached steroid discontinuation (previously not possible) were considered responders.

Results. Treatment was well tolerated and no acute or delayed toxic events were recorded. Rituximab proved to be active in 13/20 patients, with 9 complete and 4 partial responses. In 10/13 (77%) the response (platelet level > 50×10^9/L) was prompt, being achieved already after the first of the four planned infusions. After a median follow-up of 180 days (range: 60-480) 4 patients had relapsed. Age $\leq$ 60 years was correlated with a better response rate (p=0.03). No correlation was observed between response and gender, time from diagnosis to treatment (< 12 vs > 12 months), total and CD20$^+$ lymphocyte count, level of CD20 expression on B cells before the therapy and pharmacokinetics of the drug.

Interpretation and Conclusions. Rituximab appears to be a promising immunotherapeutic agent for the treatment of autoimmune thrombocytopenias.

Key words: rituximab, B-cell depletion, immune thrombocytopenias, CD20 intensity of expression, rituximab pharmacokinetics.

Haematologica 2003; 88:538-546
http://www.haematologica.org/2003_05/538.htm

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Autoimmune thrombocytopenias represent a diverse group of diseases associated with the production of antibodies reacting against platelet antigens. They may develop as isolated thrombocytopenias, of unknown etiopathogenesis, and in that case they are called idiopathic thrombocytopenic purpura (ITP). In other cases, autoimmune thrombocytopenias develop during a lymphoproliferative disorder, more frequently chronic lymphocytic leukemia (CLL), or a systemic immune disease. Most often, the course of the disease is chronic in adult patients, showing variable levels of thrombocytopenia. In the most severe forms the presence or the risk of bleeding episodes may require splenectomy (particularly in patients with ITP) or prolonged treatments with immunosuppressive, cytotoxic or steroid drugs. However, these therapies are not always effective, they are often toxic and, in some cases, they may be contraindicated because of the patient's age or the presence of comorbid conditions. Therefore, the possibility of using new immunosuppressive agents, which are both effective and not very toxic is attractive.

Rituximab is a chimeric monoclonal antibody (IgG1/κ) directed against the CD20 antigen, expressed on the surface of normal (from pre-B lymphocyte to mature B lymphocyte) and malignant B lymphocytes.[1] The action of this drug seems to be related to a mechanism of complement-mediated cytotoxicity,[2] a cell-mediated antibody-dependent cytotoxic action[1-2] as well as to the induction of apoptosis.[3-4] Rituximab proved to be effective in the therapy of CD20-positive B-cell non-Hodgkin's lymphomas (NHL).[5-6] Initial studies on animals and, subsequently, on patients treated with rituximab showed the development of a marked (although transient) B-cell depletion from peripheral blood, bone marrow and lymph nodes, but only mild changes in the immunoglobulin and complement serum levels.[5] These results, along with a good tolerance and handling of the drug, have recently prompted the use of this agent even in autoimmune diseases. The objective was to verify the clinical efficacy resulting from a prolonged and severe B-cell depletion. Rituximab was used in autoimmune cytopenias, in particular thrombocytopenias[7-11] and hemolytic anemias,[12-16] with interesting preliminary results. However, most publications include limited case studies or anecdotal reports and therefore the actual therapeutic impact of this drug still needs to be determined.

In this report, we have indicated the results of our preliminary experience in 20 patients with autoimmune thrombocytopenia who had relapsed or been refracto-

ry to at least one conventional treatment. The objective of this study was primarily to assess the therapeutic efficacy and toxicity of the drug and, at the same time, to check the role of some clinical and biological parameters, such as the serum antibody concentrations achieved during therapy and the follow-up period, the level of CD20 antigen expression, number of circulating lymphocytes and the course of serum immunoglobulins after the therapy with rituximab. In parallel, we have summarized literature data and compared them with our own results.

Design and methods

Patients

The study enrolled 20 adult patients (age > 16 years) with active and clinically symptomatic chronic autoimmune thrombocytopenia who proved to be only temporarily responsive or refractory to at least one conventional treatment. Preliminary results in 4 of them (patients 1, 2, 3, 4) have been previously reported but their data are updated here.[17] Fifteen patients were diagnosed with ITP, 1 with idiopathic thrombocytopenia and neutropenia (ITN), 2 with thrombocytopenia and concomitant undifferentiated connective tissue disease (UCTD) and 2 with autoimmune thrombocytopenia and concomitant lymphoprolipherative disorders (CLL in one and B-cell NHL in the other). Only 2 patients with ITP had been previously splenectomized. The bone marrow was evaluated before starting rituximab in order to exclude other potential causes of thrombocytopenia (in particular, myelodysplastic syndromes).

Immunologic assessment

In order to evaluate the main immunologic modifications before and after treatment with rituximab, the following analyses were performed at baseline and then at monthly intervals: a) immunophenotypic analysis of CD3, CD4, CD8, CD19 and CD20 lymphoid markers using standard procedures. The intensity of CD20 expression was also investigated and expressed as the mean fluorescence index (MFI= the ratio between the mean fluorescence of the test sample and that of its isotypic control); b) serum levels of IgG, IgA, and IgM.

Pharmacokinetics

The pharmacokinetics of rituximab were studied in a total of 7 patients, all with ITP. In all patients, rituximab serum concentrations were determined using a previously validated enzyme-linked immunoassay (ELISA).[18] Briefly, diluted serum samples were allowed to react with purified anti-rituximab polyclonal antibody coated on a microtiter plate and with anti-human IgG labeled with horseradish peroxidase. After incubation and washings, substrate solution was added and absorbance was read at 492 nm. Rituximab concentrations in samples were determined by interpolation from a standard curve by prepared diluting a known amount of rituximab into normal human serum. Results were expressed as μg/mL. The sensitivity of the method was 2 μg/mL. Quality control samples of different concentrations of rituximab were analyzed with each analytical run and showed a coefficient of variation for precision and accuracy of <15% (acceptance criterion). Blood sampling for rituximab determination was performed before and immediately after each infusion (day + 1, day + 8, day + 15, day + 22). Additional samples were taken, whenever possible, 24 hours after the first infusion and at weekly intervals after the last infusion. Samples taken at 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). For each patient, the infusions were considered as one treatment course and analyzed for the pharmacokinetic study as one group. The pharmacokinetic parameters of rituximab were determined according to a two compartment open model, with first order rates of distribution between compartments. The following pharmacokinetic parameters were considered: CL (total body clearance), V1 (volume of the central compartment), $T_{1/2\alpha}$ (distribution half-life) and $T_{1/2\beta}$ (elimination half-life).

Treatment with rituximab

Rituximab (Mabthera, Roche S.p.a., Milan, Italy) was given intravenously at a dose of 375 mg/m^2 on days +1, +8, +15 and +22. The initial infusion rate was 50 mg/h, with subsequent infusion-rates increased up to 300 mg/h if no toxicity was seen. Patients received oral acetaminophen 500 mg and intravenous chlorphenamine 10 mg as pre-medication therapy.

Concomitant treatments

No other cytotoxic or immunosuppressive drugs were given in association with rituximab. Patients already on treatment with steroids because of a very low platelet count or active bleeding at the time of rituximab administration were allowed to continue the treatment, at a low dosage (Table 6). Nevertheless only patients who reached steroid discontinuation during or after rituximab were considered responders.

Response criteria

The response was evaluated by monitoring the platelet count every week during the first month of treatment and then at monthly intervals. The hematologic improvement was assessed by evaluating the time to response (TTR; i.e. the time necessary to reach a platelet count $\geq 50\times10^9$/L), the time to maximum response (TMR; i.e. the time necessary to reach a platelet count $\geq 150\times10^9$/L) and the duration of the response. A complete response (CR) was defined as

Table 1. Main clinical and laboratory features of patients and response to rituximab therapy.

Patients	Age/sex	Disease	Months DX -RTX	Previous therapies	Ly ($\times 10^9$/L)	CD20+ve ly ($\times 10^9$/L)	CD20 MFI	Pre-RTX Plt ($\times 10^9$/L)	R to rituximab	TTR (days)	TMR (days)	RD (days)
# 1	65/f	ITP	80	S/IG	1.7	0.340	NA	10	NR	–	–	–
# 2	65/f	ITP	100	S	2.3	0.345	99	18	NR	–	–	–
# 3	51/f	ITP	25	S/IG/SPL/EDX	3.8	NA	NA	15^S	CR	7	7	480
# 4	56/f	ITP	120	S/AZA	2.7	NA	NA	23^S	CR	21	50	120
# 5	42/f	ITP	89	S/SPL	2.3	NA	NA	26^S	CR	7	75	390+
# 6	71/m	ITP	33	S/AZA-S	1.6	0.128	365	37^S	NR	–	–	–
# 7	75/m	ITP	11	S/IG	1.9	0.057	423	33^S	NR	–	–	–
# 8	60/m	ITP	6	S/IG	0.5	NA	NA	4^S	CR	28	60	330+
# 9	26/f	ITP	72	S	3	NA	NA	25	PR	7	7	120
# 10	65/f	ITP	23	S	3.2	0.384	38	16^S	NR	–	/	–
# 11	36/f	ITP	65	S/IG	1.1	0.132	NA	55^S	CR	7	7	150+
# 12	54/f	ITP	4	S	1	NA	NA	21	NR	–	–	–
# 13	16/m	ITN	10	S/IG	1.6	0.304	158	33^S	CR	7	42	270+
# 14	58/f	UCTD	6	S/IG	2.4	0.312	192	18^S	CR	7	14	240+
# 15	62/f	UCTD	96	S/AZA	1.9	0,114	32	8	PR	60	60	150+
# 16	56/m	CLL	14	CVP/S/AZA	1.4	0.700	128	21	CR	7	7	180
# 17	26/f	B-NHL	1	S	0.5	NA	NA	17	PR	7	28	180+
# 18	56/f	ITP	264	S/Ig/S+CSA	0.7	0.054	105	18^S	NR	–	–	–
# 19	67/f	ITP	7	S	2.5	0.450	120	16^S	PR	7	42	60+
# 20	76/f	ITP	144	S	1.3	0.260	40	11^S	CR	7	21	60+

ITP: idiopathic thrombocytopenic purpura; ITN: idiopathic thrombocytopenia and neutropenia; FE: Fisher Evans syndrome; UCTD: undifferentiated connective tissue disease; CLL: chronic lymphocytic leukemia; B-NHL: B-cell non-Hodgkin's lymphoma; Months Dx to RTX: months from diagnosis to rituximab; S: steroids; IG: immunoglobulin i.v.; SPL: splenectomy; EDX: cyclophosphamide; CSA: cyclosporine-A; AZA: azathioprine; VCR: vincristine; CVP: cyclophosphamide, vincristine, prednisone; Ly: total number of peripheral blood lymphocytes; CD20+ve ly: absolute number of CD20 positive lymphocyte; CD20 MFI: CD20 mean fluorescence intensity of expression; ^{S}: while in treatment with steroids; PLT: platelets; R: response; CR: complete remission; PR: partial remission; NR: no response; TTR: time to response (see text); TMR: time to maximum response (see text); RD: response duration; NA: data not available.

a platelet level ≥ 100×10^9/L and discontinuation of the steroid therapy (if designed). A partial response (PR) was defined as a platelet level ≥ 50×10^9/L and discontinuation of the steroid therapy (if being used). A minor response (MR) was a platelet level ≥ 30×10^9/L and discontinuation of the steroid therapy (if being used). Patients with less than a MR were considered non-responders (NR).

Toxicity

Rituximab-related toxicity was assessed during the period of treatment (acute toxicity; from baseline to week 6) and during the follow-up (delayed toxicity; from week 7 to week 24). Clinical and laboratory side effects were evaluated and graded according to the WHO scale.

Statistical analysis

Comparisons between groups of patients were based on the Yates – corrected χ^2 test. All p values are two-tailed. Differences were considered statistically significant if the two-sided p values were less than 0.05.

Results

Treatment program

All patients completed the therapeutic program with the four planned administrations of rituximab.

Response to therapy

Nine (45%) patients (#3, 4, 5, 8, 11, 13, 14, 16 and 20) reached a CR (Table 1). Four more patients (20%) (#9, 15, 17 and 19) had a PR. In the patient

Table 2. Time to response to rituximab therapy; modification of platelet levels during treatment in responding patients.

Pts	*Plt (10^9/L) at baseline*	*Plt (10^9/L) at day +7*	*Plt (10^9/L) at day +14*	*Plt (10^9/L) at day +21*	*Plt (10^9/L) at day +28*	*Plt ((10^9/L) at day + 35*	*Plt (10^9/L) at day + 42*
# 3	15	148	28	152	153	165	172
# 4	20	43	44	53	67	80	88
# 5	26	52	70	82	85	76	121
# 8	4	7	12	26	51	75	87
# 9	25	164	149	39	40	45	52
# 11	55	131	74	110	108	NA	NA
# 13	33	89	117	133	109	135	241
# 14	18	94	174	218	297	359	302
# 15	8	25	19	29	24	35	45
# 16	21	204	398	194	180	175	180
# 17	17	53	76	67	89	78	80
# 19	16	85	36	52	58	68	70
# 20	11	51	58	113	145	177	281

NA: data not available.

with ITN, the neutrophil count also normalized. In 10/13 (77%) who achieved a response (#3, 5, 9, 11, 13, 14, 16, 17, 19 and 20) the TTR was prompt and was observed 7 days after beginning the therapy, before the second administration of rituximab (Table 2). In contrast, in the remaining 3 patients (#4, 8 and 15) the response was observed between the second and ninth weeks. The median TMR was 28 days from the beginning of the therapy (range: 7-75 days). At a median follow-up of 180 days (range: 60-480 days), 4/13 patients (#3, 4, 9 and 16) had relapsed between day 120 and 480 from the beginning of the therapy (Table 1), three with symptomatic thrombocytopenia requiring further treatment. Age ≤ 60 years was correlated with a better response rate (p= 0.03). No correlation was observed with gender, time from diagnosis to treatment (< 12 vs > 12 months) or concomitant treatment with steroids (Table 3).

Response in patients previously treated with high dose intravenous immunoglobulin

Eight patients (Table 1) had been previously treated with Ig and 5 of these had achieved a brief, transient response (4 CR and 1 PR with a response duration between 30 to 60 days) (Table 3). Four out of the 5 Ig responders achieved a CR after rituximab therapy while 1 had no response. One Ig non-responder achieved CR after rituximab. The duration of the response to rituximab was much longer than that to Ig (Table 4).

Modification of the lymphocyte phenotype

B lymphocytes were detected in peripheral blood by simultaneous evaluation of CD19 and CD20 antigens. B-lymphocyte depletion was seen by day 30 from the begining of the therapy in all evaluated patients and persisted for at least the following 4 months. No alterations in CD3, CD4 and CD8 positive T lymphocytes were observed.

Level of CD20 expression

The level of CD20 expression was investigated before the treatment with rituximab on peripheral blood from 11 patients and, at the same time, on bone marrow from 6 patients. The median MFI was 120 (range: 32-423) in circulating B lymphocytes and 99 (range: 36-265) in bone marrow, a result which is essentially similar to that in the peripheral blood lymphocytes of a control group of healthy subjects (MFI median value 140, range 127-187). No correlation between MFI levels and response was observed in the considered cases (Table 3).

Change in the levels of IgG, IgA, and IgM after the treatment

Data on changes in Ig serum levels after treatment are summarized in Table 5. A significant change in the serum Ig level was taken to be at least a 25% decrease or increase compared to the baseline level. Changes were related to the immunoglobulin isotype. In particular, with the exception of one case, IgA levels were unaltered. Three and six months after rituximab administration, the levels of

Table 3. Comparison between some clinical and laboratory factors and response.

	Patients	*OR (CR +PR)*	*NR*	*p*
Male	5	3	2	0.3
Female	15	10	5	
≤ 60 years	12	10	2	0.03
> 60 years	8	3	5	
≤ 12 months from diagnosis to rituximab	7	5	2	0.6
> 12 months from diagnosis to rituximab	13	8	5	
Steroids in association with rituximab	13	9	4	0.5
No steroids in association with rituximab	7	4	3	
Total lymphocyte count ≤ 1.5×10^9/L before rituximab	7	5	2	0.1
Total lymphocyte count >1.5×10^9/L before rituximab	13	8	5	
$CD20^{+ve}$ lymphocyte count ≤ 0.2×10^9/L before rituximab	5	2	3	0.4
$CD20^{+ve}$ lymphocyte count > 0.2×10^9/L before rituximab	8	5	3	
Not available	7	–	–	
CD20 MFI ≤ 120 on circulating lymphocytes	6	3	3	0.7
CD20 MFI > 120 on circulating lymphocytes	5	3	2	
Not available	9	–	–	

Table 4. Comparison of the quality and the duration of response after treatment with i.v. immunoglobulin and rituximab.

Patients	*Response to IG*	*Response duration to IG (days)*	*Response to rituximab*	*Response duration to rituximab (days)*
# 1	CR	30	NR	–
# 3	CR	Not evaluable	CR	480
# 7	NR	–	NR	–
# 8	NR	–	CR	330+
# 11	PR	30	CR	150+
# 13	CR	60	CR	270+
# 14	CR	30	CR	240+
# 18	NR	–	NR	–

IG: treatment with high dose intravenous immunoglobulin;
CR: complete remission; PR: partial remission; NR: no response.

Table 5. Modifications of Ig levels (IgG, IgA, IgM) at 3 and 6 months after the treatment with rituximab. Unchanged: < 25% decrease or increase of Ig levels compared to baseline. Decrease or increase > 25%: decrease or increase from baseline levels more then 25%.

	At month +3 from rituximab				*At month +6 from rituximab*			
	Patients	*IgG*	*IgA*	*IgM*	*Patients*	*IgG*	*IgA*	*IgM*
Decrease < 25% or unchanged	17	11 (65%)	16 (94%)	14 (82%)	9	5 (56%)	9 (100%)	6 (67%)
Decrease > 25%	17	2 (12%)	0 (0)	3 (18%)	9	2 (22%)	0 (0)	3 (33%)
Increase > 25%	17	4 (24%)	1 (6%)	0 (0)	9	2 (22%)	0 (0)	0 (0)

IgG were reduced in 2/17 (12%) and 2/9 (22%) evaluable patients (patient # 13: from 9.4 g/L at baseline to 6.8 g/L and 5.2 g/L after 3 and 6 months, respectively; patient # 14: from 10 g/L at baseline to 7 g/L and 7.5 g/L after 3 and 6 months, respectively). At the same time points the levels of IgG were increased in 4/17 (24%) and 2/9 (22%) (patient #1: from 6.8 g/L at baseline to 8.7 g/L after 3 months; patient #4: from 17 g/L at baseline to 19 g/L and 22 g/L after 3 and 6 months, respectively; patient #9: from 6.3 g/L at baseline to 12 g/L after 3 months; patient #16 from 7.4 g/L at baseline to

Table 6. Steroid tapering and discontinuation during and after rituximab therapy in responding patients.

Patient	Type of steroid	Dose (mg)	Effect (Plt x10⁹/L)	Steroid dose at baseline (mg)	Steroid usage after rituximab (dose) (months after the beginning of rituximab) +1	+2	+3	+4	+5	+6	Response to rituximab
# 3	Deflazacort	60	158	40	40	40	15	0	0	0	CR
# 5	Prednisone	12.5	26	12.5	0	0	0	0	0	0	CR
# 9	Prednisone	50	4	50	6.25	0	0	0	0	0	PR
# 11	Deflacort	7.5	55	7.5	0	0	0	0	0	0	CR
# 13	Prednisone	50	33	50	0	0	0	0	0	0	CR
# 14	Prednisone	12.5	23	12.5	6.25	2.5	2.5	0	0	0	CR
# 17	Methylprednisone	4	17	4	0	0	0	0	0	0	PR
# 19	Prednisone	12.5	55	6.25	0	0	0	–	–	–	PR
# 20	Prednisone	25	11	12.5	0	0	0	–	–	–	CR

10 g/L and 11 g/L after 3 and 6 months, respectively). After 3 and 6 months the levels of IgM were reduced in 3/17 (18%) and 3/9 (33%) evaluable patients (patient #13: from 0.6 g/L at baseline to 0.4 g/L and 0.2 g/L after 3 and 6 months, respectively; patient #14: from 1.4 g/L at baseline to 0.7 g/L at both 3 and 6 months; patient #15: from 0.9 g/L at baseline to 0.6 g/L and 0.5 g/L after 3 and 6 months, respectively). No increase in IgM level was evident. Changes in Ig levels did not appear to be associated with the hematologic response.

Pharmacokinetics of rituximab

A steady increase in the median pre- and post-infusion serum concentrations of rituximab was observed at all scheduled time points during therapy, with a slow continued decline during the post-treatment period. The median drug concentration just before the 2nd, 3rd and 4th doses was 74.7 (range: 62.6-151.9) μg/mL, 126.1 (range: 99.3-170.8) μg/mL and 204.6 (range: 179.5-323.7) μg/mL, respectively. The median total clearance (CL) and volume of distribution (V1) were 0.00487 (range 0.00345- 0.00498) L/h/m^3 and 1.5322 (range 1.5058-1.6560) L/m^3 respectively. Drug disposition was characterized by a two exponential decay, with very long median distribution and elimination half-lives (31.5 hours and 482.47 hours respectively). The concentration-time curve resulting from the average population parameters as estimated by the pharmacokinetic program is shown in Figure 1.

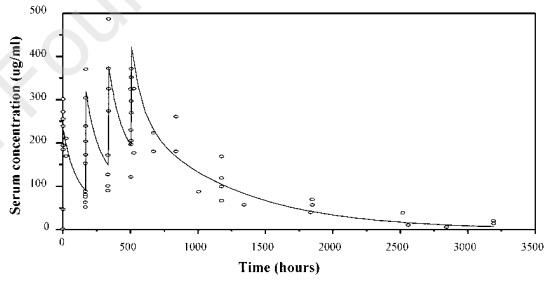

Figure 1. Concentration-time curve resulting from the average population parameters as estimated by the pharmacokinetic program.

Toxicity

During the administration of rituximab only one patient had infusional-related symptoms, with the development of urticaria, diarrhea and vomiting. No infectious complications or other significant mid-term or long-term complications were reported.

Discussion

In our experience, rituximab proved to be an effective rescue therapy for patients with severe autoimmune thrombocytopenia who did not respond to traditional treatments. Nine patients reached a CR and 4 a PR, with an overall response rate of 65%. Four out of the 13 patients who had a response relapsed at a median observation time of 180 days (range: 60-480 days), three of them requiring further therapy. A better response rate was documented in patients younger then 60 years old, while no correlation was observed between response and gender or time from diagnosis to

Table 7. Data from the literature.

Reference	*Patients*	*Disease*	*Prior SPL*	*CR*	*PR*	*OR*	*TTR weeks*	*R and SPL*		*IRT ≤2*	*Relapse*	*RD months*
								No	*Yes*			
Perotta[26]	10	ITP 8 RA 1 NHL 1	9/10	5 (50%)	1 (10%)	6 (60%)	NR	0/1	6/9	0	0/6	1+/14+
Mow[24]	1	ITP	Yes	1	–	–	NR	–	1/1	NR	0/1	7+
Grossi[23]	5	ITP 3 F.E. 2	3/5	1 (20%)	–	1 20%)	3	NR		NR	0/1	6+
Ratanatharathorn[27]	1	ITP (GVHD)	Yes	1	–	1	2	1/1		0	0/1	11+
Stasi[8,9]	32	ITP	11/32	9 (28%)	7 (22%)	16 (50%)	≤2: 10 3-5:1 ≥6: 5	10/21	6/11	18	8/16	1/27+
Saleh[7]	20	ITP	10/20	3 (15%)	2 (10%)	5 (25%)	NR	1/10	4/10	0	0/5	5+/11+
Cooper[10]	14	ITP	11/14	5 (36%)	3 (21%)	8 (57%)	≤2: 2 3-5: 3 ≥6: 3	3/3	5/11	5	0/8	3+/11+
Giagounidis[11]	12	ITP	11/12	5 (42%)	2 (17%)	7 (59%)	≤2: 6 ≥6: 1	0/1	7/11	0	1/7	NR
Rosenthal[28]	2	ITP	2/2	0	1	1	NR	1/2		NR	1/1	8
Delgado[21]	4	ITP	3/4	1	0	1	2	1/1	0/3		1	0/1 3+
Schuler[29]	1	ITP	No	0	1	1	NR	1/1		0	0/1	5+
Mehta[25]	1	SLE	Yes	–	–	0	–		0/1	0	0/1	-
Faurschou[22]	2	ITN	No	2	0	2	3-5: 1 ≥6: 1	2/2		0	0/2	10+/ 14+
Total	105	ITP 97 F.E. 3 ITN 2 RA 1 SLE 1 NHL 1	63/105	34 32%	17 16%	51 48%	≤2: 20/3 6 (55%) 3-5: 6/36(17%) ≥6: 10/36 (28%)	18/40 45%	31/60 52%	24/97 25%	10/49 22%	1-27+

ITP: idiopathic thrombocytopenic purpura; F.E.: Fisher-Evans syndrome; ITN: idiopathic thrombocytopenia and neutropenia; RA rheumatoid arthritis; SLE: systemic lupus erythematosus; NHL: non-Hodgkin's lymphoma; GVHD: graft-versus-host disease; SPL: splenectomy; CR: complete remission; PR: partial remission; OR: overall response; TTR: time to response; NR: not reported; R: response; IRT: infusion-related toxicity; RD: response duration.

treatment (< 12 vs > 12 months).

These data confirm the positive results highlighted in recent reports. So far, the therapeutic efficacy of rituximab in autoimmune thrombocytopenias has been investigated in about a hundred patients, most with ITP, and the relevant results are summarized in Table 7. A meta-analysis of the results indicates that rituximab is effective in about 50% of patients with a CR rate of 15-50%. The duration of response was quite long in a large percentage of patients, remission lasting for up to 27+ months. The percentage of recurrence was about 20%. A previous splenectomy did not appear to affect the response to the treatment while a trend towards a better outcome in younger and female patients was observed.[8] The meta-analysis indicated that a considerable percentage of patients (about 30%) had a delayed response, which could be observed even six weeks after the beginning of the therapy. In contrast, in most of our cases, the response was very prompt; in fact, 10/13 patients reached platelet levels > 50×10^9/L after the first of the four administrations of rituximab, 2/13 within the fifth week from the beginning of the treatment program and 1/13 at week nine. In our patients prednisone is unlikely to have influenced the short-term response. In fact, as previously stated, steroids were not added to rituximab but only maintained (at the same or lower dosage) in those patients who were at higher risk of bleeding (Table

6), though without leading to a significant increase in platelet counts (i.e. never higher then 55 × 10^9/L). Furthermore no substantial difference in the response rate was evident in those patients who received steroids in association with rituximab and those who did not (Table 3). The mechanism of action of rituximab, which may explain such a quick response, is yet to be elucidated. It has been postulated that it is unlikely to be due to a fall of autoantibody level; a mechanism of Fc-receptor saturation of the reticuloendothelial system by opsonized CD20-positive cells may be involved in the initial phase of response.[8] The role of B-cell depletion and its potential interference with the production of autoantibodies is probably more important in cases of delayed response and in the maintenance of the response.

The objective of the pharmacokinetic study was to assess the serum levels of rituximab in a group of patients with fewer B cells than found in subjects with NHL. The rituximab disposition in such a group appears to be similar to that observed in lymphoma patients.[18,19] Despite previous pharmacokinetic studies having demonstrated an association between clinical response and serum rituximab accumulation both during and after treatment,[20] in our group of patients no difference was observed in serum levels between responders and non-responders.

The level of CD20 antigen expression (MFI) in B-cell NHLs proved to be a factor predicting the response to treatment with rituximab.[2] In our cases of ITP, the MFI levels of the CD20 in peripheral blood and bone marrow B-lymphocytes were heterogeneous with a median expression of 120 (range: 32-423) and 99 (range: 36-265) respectively, substantially similar to levels observed in the peripheral blood lymphocytes of a control group of healthy subjects (MFI median value 140, range 127-187). No correlation between MFI levels of CD20 and clinical response was observed.

After treatment with rituximab, a depletion of circulating B lymphocytes was observed in all responsive and unresponsive patients. Therefore, the degree of B-cell depletion in peripheral blood did not predict response. In contrast, serum Ig levels changed in relation to patients and different immunoglobulin isotypes. However, no correlation between the change in Ig levels and the clinical response was observed.

The treatment with rituximab was particularly well tolerated. In fact, only one patient had symptoms associated with the release of cytokines. The analysis of literature data (Table 4) indicates that during the infusion of rituximab roughly 25% of patients experienced symptoms associated with the release of cytokines, although these symptoms were not serious in any case. It is likely that the smaller number of B cells in these patients than in patients with NHL is the main explanation of this fact. Furthermore, in our experience, there were no signs of mid-term and long-term toxicity and, in particular, no significant infectious events were recorded. In this regard, it is necessary to stress that, despite the systemic B-lymphocyte depletion from peripheral blood, the serum level of IgG had increased (24% of patients) rather than decreased (12% of patients) by 3 months after the rituximab therapy. Discontinuation of chronic steroid therapy or other immunosuppressive treatments perhaps underlies this finding in some patients.

In conclusion, rituximab seems to be an effective agent for the treatment of autoimmune thrombocytopenias. It seems to be very well tolerated and effective in about 50% of subjects in the short and medium term. Most post-treatment follow-ups are still short and consequently the actual impact of this therapy in the long-term awaits further definition. This aspect seems to be particularly important, especially in the perspective of considering this treatment as an alternative to splenectomy. Clinical and biological predictive factors of response remain to be determined and mechanisms of resistance should still be elucidated.

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Pre-publication Report & Outcomes of Peer Review

Contributions

FZ and NV: responsible for the conception and design of the study, analysis and interpretation of data, writing of the manuscript. II: performed and interpreted all pharmacokinetic data. AS, SDV, MT, VT, MT, ALM: participated in the patients' care and contributed to the interpretation of the results. PM: performed immunophenotypic analysis. AZ, MB and RF were the Head of Departments in which the patients were treated. All authors gave their final approval to the study. Primary responsibility for the paper and for all tables: FZ; primary responsibility for Figure 1: IA.

Disclosures

Conflict of interest: none.

Redundant publications: yes, < 50%.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Martino Introna, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Introna and the Editors. Manuscript received November 27, 2002; accepted March 11, 2003.

In the following paragraphs, the Associate Editor summarizes the peer-review process and its outcomes.

What is already known on this topic

Immune thrombocytopenias are caused and maintained by a still largely elusive mechanism mediated by auto-antibodies. Treatments are largely symptomatic and determined by the overall evaluation of the risk in each individual patient. Several reports have suggested the possible use of the chimeric anti-CD20 monoclonal antibody, rituximab, in ITP.

What this study adds

In 20 ITP patients with active disease which had relapsed or was refractory to standard therapies, the effect of rituximab was evaluated by measurement of platelet counts. By this criteria 13/20 patients achieved a measurable response.

Caveats

Experimental treatments should be applied when life-threatening hemorrhage risk is present and not on the basis of platelet counts alone. Further research addressing relevant end points (severity of bleeding), quality of life and economic considerations is recommended.