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RESEARCH ARTICLE

The impact of rituximab infusion protocol on the long-term outcome in anti-MuSK myasthenia gravis

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Abstract

Objective: To evaluate whether the clinical benefit and relapse rates in anti-muscle-specific kinase (MuSK) myasthenia gravis (MG) differ depending on the protocol of rituximab followed. **Methods**: This retrospective multicentre study in patients with MuSK MG compared three rituximab protocols in terms of clinical status, relapse, changes in treatment, and adverse side effects. The primary effectiveness endpoint was clinical relapse requiring a further infusion of rituximab. Survival curves were estimated using Kaplan–Meier methods and survival analyses were undertaken using Cox proportional-hazards models. **Results**: Twenty-five patients were included: 11 treated with protocol 4 + 2 (375 mg/m^2/4 weeks, then monthly for 2 months), five treated with protocol 1 + 1 (two 1 g doses 2 weeks apart), and nine treated with protocol 4 (375 mg/m^2/4 weeks). Mean follow-up was 5.0 years (SD 3.3). Relapse occurred in 18.2%, 80%, and 33.3%, and mean time to relapse was 3.5 (SD 1.5), 1.1 (SD 0.4), and 2.5 (SD 1.4) years, respectively. Based on Kaplan–Meier estimates, patients treated with protocol 4 + 2 had fewer and later relapses than patients treated with the other two protocols (log-rank test $P = 0.0001$). Patients treated with protocol 1 + 1 had a higher risk of relapse than patients treated with protocol 4 + 2 (HR 112.8, 95% CI, 5.7–2250.4, $P = 0.002$). Patients treated with protocol 4 showed a trend to a higher risk of relapse than those treated with protocol 4 + 2 (HR 9.2, 95% CI 0.9–91.8, $P = 0.059$). **Interpretation**: This study provides class IV evidence that the 4 + 2 rituximab protocol has a lower clinical relapse rate and produces a more durable response than the 1 + 1 and 4 protocols in patients with MuSK MG.

Introduction

Myasthenia gravis (MG) is an autoimmune disease caused by antibodies to antigens of the postsynaptic neuromuscular junction and clinically characterized by fatigable muscle weakness.[1–5] Approximately, 5% of MG patients have autoantibodies against muscle-specific kinase (MuSK),[6,7] and the frequency is higher if an IgG-specific MuSK-cell-based assay is used to detect these antibodies.[8] MuSK antibody titers are mainly of the IgG4 subclass and considered to correlate with patients' clinical status.[9,10] Patients with MuSK MG often present early, severe bulbar and respiratory involvement. Furthermore, they have a poorer response to acetylcholine esterase inhibitors, standard immunosuppressant therapies, and intravenous immunoglobuline than MG patients without MuSK.[10] For these reasons, the need for an efficient drug to treat patients with MuSK MG has long been awaited.

Rituximab, a monoclonal antibody that targets the CD20 antigen in B cells, was first used to treat non-Hodgkin's B-cell lymphoma.[11–13] However, it later emerged as a highly effective tool to manage autoimmune diseases.[14,15] As autoreactive B cells have a clear pathogenic role in the development of MG, rituximab has been used in drug-resistant MG patients and several authors have described its benefits.[16–20] Rituximab has proven to be more effective and to have a longer clinical benefit in patients with antibodies against MuSK[17,20] than in patients with anti-acetylcholine receptor (AChR)-positive MG.

Rituximab has also shown to be a remarkably effective drug in other IgG4-mediated diseases of the central and peripheral nervous system and connective tissue of the skin or kidneys, such as CIDP with anti-Contactin-1 and anti-Neurofascin-155 antibodies,[21–23] pemphigus,[24] membranous glomerulonephritis,[25] and LGI1 limbic encephalitis.[26–28] All IgG4-mediated disorders seem to share similarities in epitope binding, human leukocyte antigen associations, disease mechanism, and underlying etiology, thus explaining the extraordinary response to rituximab observed in all such disorders.[29]

We previously reported the benefits of rituximab in a large series of drug-resistant patients (11 AChR-positive MG and 6 MuSK-positive MG). Remarkably, all six patients with MuSK MG achieved minimal manifestations (MM) or a better Myasthenia Gravis Foundation of America post-intervention status (MGFA PIS), and no additional infusions were required during a 31-month follow-up (4–60). The protocol we used was 375 mg/m^2 weekly for 4 weeks, then monthly for 2 months (4 + 2).[17]

Infusion protocols for rituximab in autoimmune disorders[14,17–25,30] differ, and no protocol has yet been established for rituximab in MuSK MG. We wondered whether our treatment regimen contributed to the excellent long-term outcome of MuSK MG. Here, we describe the clinical follow-up in 25 patients with MuSK MG treated with different infusion protocols of rituximab. We report the clinical effectiveness, adverse effects, and differences in clinical outcomes in patients treated with the three most commonly used rituximab treatment protocols.

Methods

Patients and clinical evaluation

In this multicentre, retrospective observational study, we included all patients with MuSK MG treated with rituximab at 11 hospitals in Spain between January 1, 2006 and March 31, 2016. We collected demographic and clinical data at onset and during follow-up. Patients were followed until November 30, 2017. To assess the clinical response, the MGFA PIS was determined periodically, and relapse rate and changes in treatment were recorded. The primary effectiveness endpoint was clinical relapse requiring a further infusion of rituximab. Safety was evaluated based on the effects of rituximab treatment on mortality and morbidity.

Treatment protocol

The standard treatment protocol for patients with MuSK MG is first-line prednisone and second-line immunosuppressors (azathioprine/mycophenolate followed by cyclosporine) if the response is not adequate or if the dose of prednisone required is higher than 40 mg every other day. Patients were considered drug-resistant when no significant clinical improvement was achieved after prednisone and at least two second-line immunosuppressants.[31] Patients with MGFA IV or V and no response to prednisone were treated with rituximab as a second-line drug in accordance with our previous results.[17] Rituximab was administered following one of three protocols: (4 + 2) 375 mg/m^2 every week for four consecutive weeks and then monthly for the next 2 months; (1 + 1) two 1 g doses separated by 2 weeks; and (4) 375 mg/m^2 every week for four consecutive weeks. Rituximab re-infusions were administered only if patients relapsed. A relapse was defined as the reappearance of myasthenic symptoms that limited daily activity.

Protocol approval and patients' informed consent

Informed consent was obtained from all patients and the study was approved by the ethics committees at all

participating hospitals. Permission for compassionate use of rituximab was given by the Spanish Ministry of Health.

Statistical analysis

A descriptive data analysis was performed. Demographic characteristics are reported as means and standard deviations (SD) for quantitative variables and as percentages for categorical variables. Differences between patient subgroups in baseline characteristics were evaluated using ANOVA to compare categorical variables and the Mann–Whitney *U* test to compare quantitative variables. Log-rank tests were used to compare Kaplan–Meier estimates for survival curves for the three treatment groups in a time-to-first-event analysis. Hazard ratios and 95% confidence intervals were estimated using a Cox proportional-hazards model. Survival time was from the date of the first rituximab dose to date of reinfusion due to clinical relapse. Patients with no relapse were censored at date of last follow-up. Data analysis was carried out using Stata 13.0 (StataCorp, College Station, TX) for Windows.

Results

Twenty-five patients with MuSK MG were included in the study: 11 were treated with protocol 4 + 2, five were treated with protocol 1 + 1, and nine were treated with protocol 4. Table 1 summarizes the demographic and clinical characteristics of the three treatment groups. The only significant difference between groups in baseline characteristics in the univariate analyses was age at onset ($P = 0.035$). Age at which rituximab was started did not differ significantly between groups ($P = 0.449$). Treatment with rituximab was associated with a significant improvement in all patients, and all achieved MM or a better MGFA PIS. After rituximab was started, other treatment, especially prednisone, was decreased or withdrawn in all patients (Table 2). Patients were followed up for a mean of 5.0 years (SD 3.3).

No patient presented severe adverse events. During the infusion, seven patients presented mild symptoms: three of the 11 patients in the 4 + 2 protocol group (one facial paresthesias, one fever, and one skin and mucous itching); one of the five patients in the 1 + 1 protocol group (mild gastrointestinal symptoms); and three of the nine patients in the protocol 4 group (two patients with skin rash and one fatigue). All these symptoms disappeared when premedication with antihistamine and steroid treatment was given before further infusions of rituximab.

Relapse occurred in two patients in group 4 + 2, in four patients in group 1 + 1, and in three patients in group 4, resulting in relapse rates of 18.2%, 80%, and 33.3%, respectively. The mean time to relapse was 3.5 years (SD 1.5, Min 2.5–Max 4.6), 1.1 years (SD 0.4, Min 0.7–Max 1.6), and 2.5 years (SD 1.4, Min 1.2–Max 4.1), respectively. Kaplan–Meier estimates showed a clear difference in survival curves among groups, with patients treated with protocol 4 + 2 having fewer relapses (log-rank test $P = 0.0001$). Survival curves are shown in Figure 1.

Table 1. Demographic and clinical data of patients included in the study and comparison between groups.

	4 + 2 doses ($n = 11$)	1 + 1 ($n = 5$)	4 doses ($n = 9$)	*P*
Age at onset (years)				
Mean (SD)	47.2 (15.7)	39.5 (17.4)	30.5 (9.6)	0.035
Min–Max	14.7–73.8	18.3–65.9	17.0–45.9	
Sex (% female)	11 (100%)	4 (80%)	9 (100%)	0.200
Worst MGFA class	IIIB 3 (27.3%)	IIB 2 (40%)	IIIB 6 (66.7%)	0.073
	IVB 6 (54.5%)	IIIB 1 (20%)	IVB 1 (11.1%)	
	V 2 (18.2%)	IVB 1 (20%)	V 2 (22.2%)	
		V 1 (20%)		
Age when RTX was started (years)				
Mean (SD)	55.4 (12.5)	46.3 (21.9)	49.2 (16.5)	0.449
Min–Max	35.3–78.9	19.4–78.9	26.5–70.1	
Best MGFA PIS	CRS 5 (45.4%)	CSR 1 (20%)	CSR 1 (11.1%)	0.335
	PR 2 (18.2%)	PR 3 (60%)	PR 2 (22.2%)	
	MM 4 (36.4%)	MM 1 (20%)	MM 6 (66.7%)	
Follow-up (years)				
Mean (SD)	6.6 (3.5)	3.5 (1.9)	4.0 (3.3)	0.114
Min–Max	1.4–11.6	1.3–6.3	1.6–11.7	
MGFA PIS last visit	CRS 4 (36.4%)	CRS 2 (40%)	CSR 1 (11.1%)	0.665
	PR 1 (9.1%)	PR 1 (20%)	PR 2 (22.2%)	
	MM 6 (54.6%)	MM 2 (40%)	MM 6 (66.7%)	

Table 2. Previous treatments, treatment before rituximab, and treatment at last evaluation for each patient. Time regarding how long treatment was provided both for treatment before rituximab and treatment at last visit are provided in brackets.

Patient	Rituximab protocol	Previous treatments	Treatment before rituximab (treatment duration)	Treatment at last visit (treatment duration)
1	4 + 2	Pyridostigmine, PDN, IVIG	Pyridostigmine 300 mg per day (6 months), PDN 60 mg per day (1 month)	None (53 months)
2	4 + 2	Pyridostigmine, DFZ, CYA	DFZ 30 mg EOD (99 months), CYA 150 mg every 12 h (87 months), Pyridostigmine 300 mg per day (99 months)	DFZ 6 mg EOD (61 months), Pyridostigmine 240 mg per day (61 months)
3	4 + 2	PDN, IVIG, CYA	PDN 60 mg/5 mg EOD (4 months), CYA 100 mg every 12 h (2 months)	None (59 months)
4	4 + 2	PDN, AZA, MMF, CYA, IVIG	PDN 65 mg EOD (48 months), CYA 150 mg per day (9 months)	PDN 10 mg EOD (23 months)
5	4 + 2	PDN, IVEG, PLEX, Pyridostigmine	PDN 60 mg per day (2 months)	PDN 40 mg EOD (17 months)
6	4 + 2	PDN, THYM, AZA, IVIG, CPH, CYA	Pyridostigmine if needed (38 months) (CPH abandoned 19 months before)	None (24 months)
7	4 + 2	THYM, PDN, AZA, CPH, IVIG, MMF	MMF 2 g per day (3 months)	None (96 months)
8	4 + 2	PDN, THYM, AZA, MMF, CYA, IVIG, CPH	PDN 15 mg EOD (5 months, 245 months in total with PDN), MMF 2 g per day (3 months)	PDN 30 mg EOD (18 months)
9	4 + 2	PDN, AZA	PDN 40 mg EOD (19 months, 79 months in total with PDN)	None
10	4 + 2	PDN, AZA, IVIG, CYA, PLEX	PDN 30 mg EOD (18 months, 51 months in total with PDN), CYA 125 mg per day (6 months)	Pyridostigmine if needed (30 months)
11	4 + 2	PDN, AZA, CYA, IVIG, Pyridostigmine	PDN 80 mg EOD (28 months), Pyridostigmine 300 mg per day (29 months)	PDN 7 mg EOD (3 months)
12	1 + 1	Pyridostigmine, PDN, MMF	PRD 60 mg EOD (1 month), Pyridostigmine if needed	PDN 10 mg EOD (7 months)
13	1 + 1	PDN	PDN 25 mg EOD (6 months), Pyridostigmine if needed	None (12 months) (rituximab when relapse)
14	1 + 1	PDN	PDN 60 mg/30 mg EOD (4 months)	None (35 months)
15	1 + 1	PDN, AZA, MMF	PDN 40 mg per day (1 month, 223 months in total with PDN)	PDN 12.5 mg per day (29 months)
16	1 + 1	IVIG, PDN, MMF, PLEX, CYA	PDN 45 mg EOD (47 months), CYA 100 mg every 12 h (10 months), Pyridostigmine 240 mg every day (11 months)	Pyridostigmine if needed (21 months)
17	4	THYM, Pyridostigmine, PDN, TAC, PLEX	PDN 5 mg EOD (60 months), TAC 2 mg every 12 h (10 months), Pyridostigmine 120 mg per day (96 months)	None (20 months)
18	4	PDN, THYM, IVIG, AZA	PDN 20 mg per day (36 months), MMF 1 g per day (24 months)	MMF 1.500 g per day (30 months)
19	4	Pyridostigmine, PDN, AZA, TAC, IVIG, PLEX	TAC 1, 5 mg every 12 h (23 months), PDN 20 mg EOD (23 months), Pyridostigmine 180 mg per day (35 months)	CYA 125 mg every 12 h (23 months), PDN 50 mg EOD (23 months)
20	4	Pyridostigmine, PDN, AZA, PLEX, IVIG, THYM	PDN 30 mg EOD (264 months), Pyridostigmine 360 mg per day (265 months)	PDN 10 mg per day (45 months)
21	4	Pyridostigmine, PDN, AZA, PLEX, IVIG, THYM	PDN 30 mg per day (300 months)	PDN 10 mg per day (53 months)
22	4	Pyridostigmine, PDN, AZA, PLEX	AZA 111 mg per day (60 months)	None (21 months)
23	4	Pyridostigmine, PDN, AZA, IVIG, PLEX	AZA 111 mg per day (60 months), PDN 30 mg EOD (120 months)	PDN 10 mg EOD (26 months)
24	4	Pyridostigmine, PDN, AZA, MMF	MMF 2.5 g per day (45 moths), PDN 30 mg/15 mg EOD (71 months)	PDN 10 mg EOD (12 months)
25	4	PDN, MTX, AZA, MMF, IVIG, PLEX	PDN 70 mg per day (106 months)	None (116 months)

PDN, prednisone; DFZ, Deflazacort; AZA, azathioprine; MMF, Mycophenolate mofetil; CYA, cyclosporine; TAC, Tacrolimus; CPH, cyclophosphamide; MTX, methotrexate; IVIG, intravenous immunoglobulin; PLEX, plasma exchange; THYM, thymectomy; EOD, Every other day.

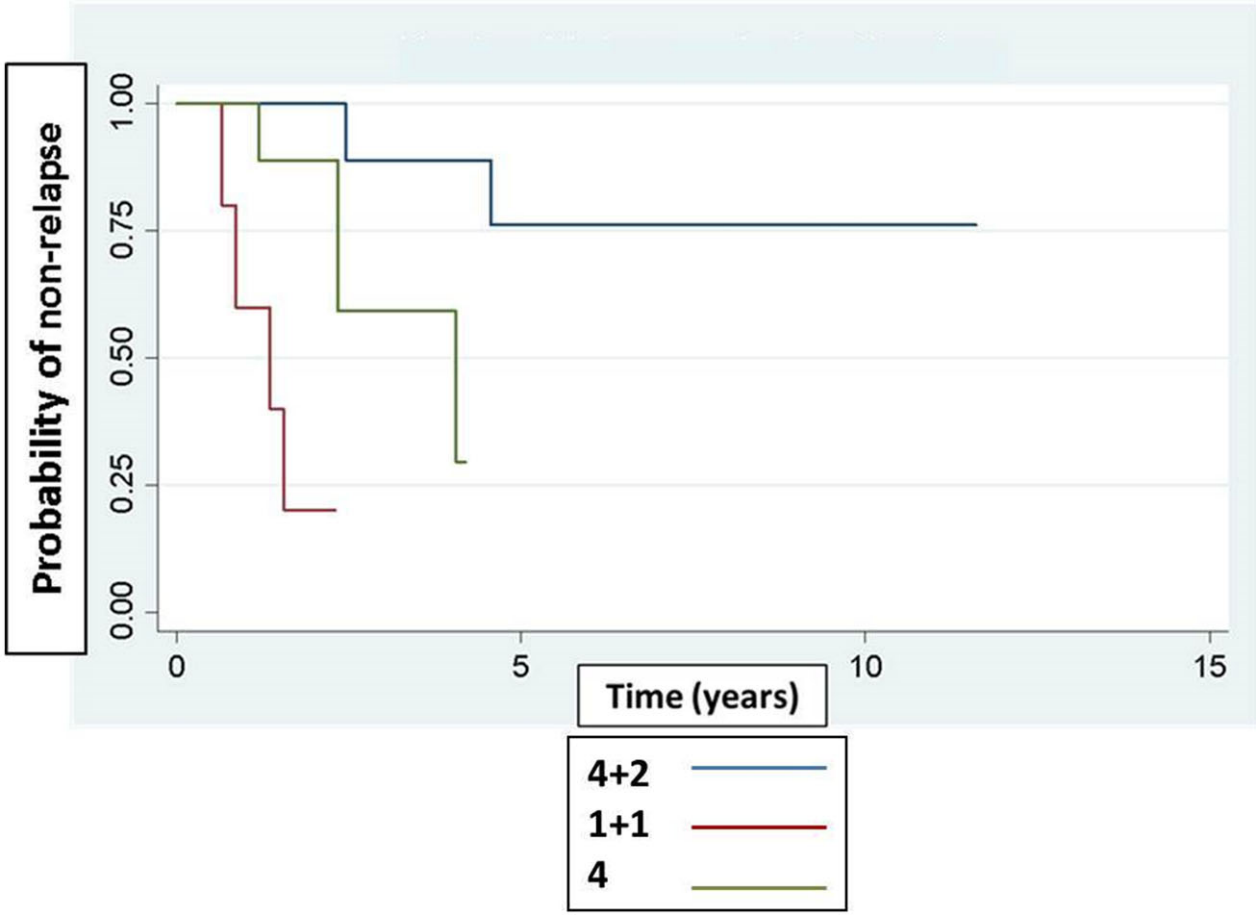

Figure 1. Kaplan–Meier survival curves for relapse in each group of patients with MuSK MG according to the rituximab protocol followed: (4 + 2) 375 mg/m^2 every week for four consecutive weeks and then monthly for the next 2 months; (1 + 1) two 1 g doses separated by 2 weeks; and (4) 375 mg/m^2 every week for four consecutive weeks.

The Cox proportional-hazards regression model showed that patients treated with protocol 1 + 1 had a higher risk of relapse and a greater need for reinfusion with rituximab than patients treated with protocol 4 + 2 (hazard ratio [HR] 112.8, 95% confidence interval [CI], 5.7–2250.4, $P = 0.002$). Patients treated with protocol 4 also showed a trend to a higher risk of relapse than patients treated with protocol 4 + 2 (HR 9.2, 95% CI 0.9–91.8, $P = 0.059$) (likelihood ratio test = 15.1, $P = 0.0005$).

Discussion

Rituximab has previously shown level IV evidence as a beneficial and durable treatment option for drug-resistant MuSK MG.[17,20] Our results further add that the treatment protocol plays a key role in reducing clinical relapse and achieving a long-lasting response. All patients included in this study improved after rituximab treatment and all patients achieved MM or a better MGFA PIS and a long-lasting response after an extended follow-up. Moreover, prednisone and other immunosuppressive therapies were withdrawn or tapered to lower doses in all cases. Rituximab was also found to be safe as no patient developed severe side effects. The frequency of mild-moderate adverse events did not differ between the three groups. This finding is of particular note because most patients had been treated previously with two or more immunosuppressive therapies. Close follow-up is recommended, however, as several cases of side effects of rituximab have been reported in patients with MG.[32]

Various protocols of rituximab have been published to treat patients with MuSK MG, and relapse rates and durability of response have differed.[17,20] As no infusion regimen has yet been standardized in this setting, we compared the clinical response of 25 MuSK MG patients treated with three different rituximab protocols. Because all patients responded to the drug, the primary effectiveness endpoint chosen was the appearance of a clinical relapse requiring a further infusion of rituximab. Based

on Kaplan–Meier estimates, the most efficient protocol was the 4 + 2 protocol. It showed the lowest relapse rate (18.2%) and the longest time to relapse after an extended follow-up period. The Cox proportional-hazards regression confirmed that patients treated with protocol 1 + 1 had a significantly higher risk of relapse and greater need for reinfusion with rituximab than patients receiving the 4 + 2 protocol. Patients treated with the protocol based on four doses also showed a trend toward a significantly higher risk of relapse than those treated with protocol 4 + 2.

Although prominent B-cell pathology has been described in MuSK MG,[33–35] the mechanism by which IgG4 antibodies are produced remains unclear. Rituximab works by depleting pre-B cells and mature and memory B cells from the circulation. Exactly how this leads to antibody reduction, however, is still to be determined as B-cell populations that do not express CD20 produce a considerable portion of circulating immunoglobulin.[36] Why some patients relapse and other do not is unclear. A recent study of autoantibody-producing cells during disease relapse in three MuSK MG subjects who had previously achieved rituximab-induced remission revealed autoantibody-expressing $CD27^+$ B plasmablasts within the reconstituted repertoire during relapse but not during remission or in controls.[37] The authors proposed that MuSK-specific memory cells continuously supply a population of short-lived, autoantibody-secreting plasmablasts, and suggested that rituximab works by indirectly depleting the CD20− plasmablast population by diminishing $CD20^+$ memory B cells. Alternatively, they hypothesized that rituximab could work by directly depleting a fraction of plasmablasts that may be $CD20^+$.

In our study, we observed that all patients with MuSK MG treated with rituximab had a beneficial response. However, the relapse rate differed depending on the posology used, with the 4 + 2 protocol being the most effective in terms of response durability. We hypothesize that this protocol is more efficacious in depleting the pool of plasmablasts-progenitor $CD20^+$ memory B cells or the subset of $CD20^+$ plasmablasts than the two other protocols followed. Further studies are needed to confirm this hypothesis.

The main limitation of our study is the small number of patients. MuSK MG is a rare disorder, however, and as not all cases are severe or drug-resistant, rituximab is not always required. The strengths of the study are the significant results in the statistical analysis, and particularly the long clinical follow-up, with an overall mean of 5 years.

In summary, our findings add to the evidence that rituximab is effective and safe in the treatment of MuSK MG. We recommend treating patients with a sole induction regimen of rituximab following the protocol 4 + 2 (375 mg/m^2 every week for 4 consecutive weeks and then monthly for the next 2 months), since this protocol ensures a minimal rate of clinical relapse and a long-lasting response to rituximab. To minimize potential adverse events, we recommend re-treating patients with rituximab in cases of clinical relapse only.

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Conflicts of Interest

None.

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