International Immunopharmacology 73 (2019) 94–97

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Comparison between the efficacy of intralesional rituximab versus intralesional triamcinolone in the treatment refractory Pemphigus Vulgaris lesions: A randomized clinical trial

Fariba Iraji, Farzaneh Danesh, Gita Faghihi*, Amirhossein Siadat, Fatemeh Mokhtari, Mahboobeh Talakoob, Hossein Hafezi

Department of Dermatology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Keywords:
Pemphigus Vulgaris
Rituximab
Triamcinolone

ABSTRACT

Background: Pemphigus Vulgaris (PV) is a vesiculobullous autoimmune disorder characterized by production of autoantibody against cellular adhesion molecules. The treatment of PV is based on the use of systemic corticosteroid along with immunosuppressive therapy, but sometimes there are limited resistant lesions not responding to conventional systemic therapy. This double-blind, randomized clinical trial was designed to evaluate the efficacy of intralesional rituximab versus triamcinolone in treatment of the refractory scalp and mucosal pemphigus lesions.

Methods: 2 refractory lesions of PV were selected in 21 patients, and they were randomly assigned to two groups to be treated with either intralesional triamcinolone or rituximab for 2 times at one-month interval. All of the patients were under treatment with prednisolone and azathioprine. Patients were visited at the baseline, 1 and 6 months after treatment, and all information including demographic characteristics of the patients, Pemphigus Vulgaris Lesion Severity Score (PVLSS), Epithelialization Index (EI) and patient's satisfaction (using Visual Analogue Scale (VAS)) were obtained. The collected data were analyzed using SPSS software (ver18).

Results: The results showed that, both rituximab and triamcinolone were effective in treatment of the refractory PV lesions ($p < 0.05$). However, there was no significant difference between the effect of intralesional rituximab and triamcinolone ($p > 0.05$). In addition, no side effect was observed in both groups.

Conclusion: Regarding the results of the present study, the use of intralesional rituximab can be suggested for treatment of the resistant PV lesions as an alternative to intralesional triamcinolone or using more aggressive systemic therapy.

1. Introduction

Pemphigus Vulgaris (PV) is a rare disease with an incidence varying from 0.76 to 16.1 cases per million people in Finland and Ashkenazy Jewish population, and an incidence of 1 case per 100,000 people in Iran [1,2]. PV is a potentially fatal and chronic autoimmune disease characterized by mucocutaneous blisters and erosions resulting from the autoantibody against desmoglein (Dsg) 1 and 3 [3]. Desmoglein is a cell adhesion molecule attaching keratinocytes. Desmoglein 1 and desmoglein 3 are mainly located in the oral cavity and the skin respectively. In the PV, autoantibodies separate keratinocytes through Dsg destruction, named as acantholysis, which in turn causes vesicles and bullae both on the skin and oral mucosa [4–6].

Systemic steroids, cyclophosphamide, azathioprine, methotrexate, antagonists of Tumor Necrosis Factor α (TNF-α), plasmapheresis and Intravenous Immune Globulin (IVIG) are the most common treatments used for treating PV [7–11]. In patients who are refractory to routine treatments, rituximab (a humanized antibody against CD20) is tried [12].

Rituximab therapy results in a decrease in the number of B cells, along with Dsg-specific T cells. Many serious side effects have been attributed to the use of systemic rituximab [13]. Also, many oral and scalp lesions of PV may be resistant to standard treatments. In this regard, the current study was aimed to evaluate the efficacy of intralesional rituximab to reduce the risk of side effects and increase the possibility of healing the resistant oral and scalp lesions. In this double-blind, randomized clinical trial, the efficacy of intralesional rituximab was compared versus triamcinolone in treatment of the refractory scalp

* Corresponding author.
E-mail address: g_faghihi@med.mui.ac.ir (G. Faghihi).

https://doi.org/10.1016/j.intimp.2019.04.031
Received 9 February 2019; Received in revised form 14 April 2019; Accepted 15 April 2019
Available online 10 May 2019
1567-5769/

and mucosal PV lesions.

2. Materials and methods

2.1. Subjects

In this randomized clinical trial study, 21 patients with PV (including15 males and 6 females, with mean age of 52.38 ± 13.49 years old (mean ± SD), and age range from 29 to 75 years old) participated. The duration of PV in two groups ranged between 8 months and 10 years. All of the participants were concurrently treated with systemic steroids (prednisolone 5–60 mg/day) and azathioprine (2–3 mg/day). This study was performed in clinics affiliated with Isfahan University of Medical Sciences, Isfahan, Iran during 2017–2018, and it was approved by the Ethical Committee of Isfahan University of Medical Sciences and was also registered in the Iranian Registry of Clinical Trial (IRCT20181224042105N1). Informed consent was obtained from all participants before entering the study.

The diagnosis of PV was established using clinical, histological and immunofluorescence characteristics. Patients with the following criteria were included in this study: a) lack of response to oral immunosuppressive treatment after 4 months and the presence of at least 2 almost same-sized oral or scalp lesions; b) non-infected lesions; c) absence of rituximab contraindications. Contraindications of rituximab included allergy to it or its components, hepatitis B carrier, cardiac arrhythmia, angina pectoris, high tumor burden, and active infection. Allergic reaction to rituximab and infection at the site of the lesion were considered as exclusion criteria.

All information including demographic characteristics of the patients, Pemphigus Vulgaris Lesion Severity Score (PVLSS), Epithelialization Index (EI) and patient's satisfaction (using Visual analogue Scale (VAS)) were obtained and recorded [14,15]. Two almost same-sized uninfected lesions were selected in the scalp or oral cavity in each patient and, they were randomly assigned to two groups to receive either intralesional injection of 10 mg/ml of rituximab or 10 mg/ml of triamcinolone. Totally, 42 lesions with either were treated either with rituximab or triamcinolone. In the case of non-healing, the lesion was treated again with the same medication at 1-month interval only once. Patients were visited at the baseline, 1 and 6 months after treatment, and the size and characteristics of the lesions, PVLSS and VAS were all recorded.

2.2. Statistical analysis

Data analysis was carried out using SPSS software (ver18; SPSS Inc. Chicago, IL, USA). The normality of data was determined by Kolmogorov-Smirnov test, and non-parametrical, Chi-Square and Fisher's exact tests were used to evaluate the associations. The p-value of < 0.05 was considered as statistically significant.

3. Results

Totally, 42 lesions were selected in the oral cavity or scalp of the patients, and they were randomly assigned to two groups and received either intralesional triamcinolone or rituximab (Table 1).

Table 1
Characteristics of patients and frequency of lesions in each site.

	Frequency	
Age (mean ± SD)	52.38 ± 13.49	
Sex (N = 21)	Male	15 (71.4%)
	Female	6 (28.6%)
Lesions involvement (N = 42)	Scalp	22 (52.3%)
	Oral cavity	15 (35.7%)
	Lip	5 (12%)

There was no significant difference between the mean size of lesions before treatment ($p = 0.19$) (Table 2).

3.1. Comparison of the size of lesions before and after treatment with rituximab

Statistical analysis showed a significant difference in the mean size of lesions before and at 1 and 6 months after the injection of rituximab ($p < 0.001$ for both groups) (Table 2). However, there was no statistically significant difference in the mean size of lesions at 1 and 6 months after injection ($p = 0.065$) (Table 2). Also, the patient's satisfaction using VAS score was significantly different before and after treatment in the rituximab group ($p < 0.001$). Moreover, in 2 patients lesions treated with rituximab relapsed after the second injection, but remitted at the end of 6 months.

3.2. Comparison of the size of lesions before and after treatment with triamcinolone

Statistical analysis showed a significant difference in the mean size of lesions before and at 1 and 6 months after the injection of triamcinolone ($p < 0.001$ for both groups) (Table 2), However, there was no statistically significant difference in the mean size of lesions at 1 and 6 months after injection ($p = 0.16$). Also, the patient's satisfaction using VAS score was significantly different before and after treatment in the triamcinolone group ($p < 0.001$) (Table 2).

3.3. Comparison between rituximab and triamcinolone groups

There was no significant difference in the mean size of lesions after 1 and 6 months of treatment with rituximab and triamcinolone ($p = 0.28$ and $p = 0.71$, respectively) (Table 3).

PVLSS and VAS were also analyzed before and after treatment (Tables 3 and 4). Moreover, EI was compared after treatment.

3.4. Side effects

No side effect was observed in both groups. The burning sensation was found to be a little more in the rituximab group.

The majority of the study patients had only two refractory lesions, and for others who had a few lesions, refractory lesions did not resolve until the end of the follow-up period (i.e. 6 months), since the injection of drugs had not been done, but according to the concept of our approved proposal, they were not considered to be evaluated regarding their parameters.

4. Discussion

The present study was aimed to evaluate the efficacy of intralesional rituximab and triamcinolone for the treatment of refractory oral and scalp lesions. The results showed that, treatment with both rituximab and triamcinolone decreased the size of PV lesions, but there was no statistically significant difference in terms of the size of PV lesions between the two treatments.

Rituximab is a chimeric monoclonal antibody against CD20, capable of depleting $CD20^+$ B cells through increasing apoptosis [16]. The previous studies showed that rituximab therapy is useful for treatment of different autoimmune diseases such as rheumatoid arthritis, vasculitis, Sjogren syndrome and SLE [17–20] as well as PV. In a study by E. Schmidt et al., rituximab was injected for patients with PV, and their results showed that, the injection of rituximab induced long-standing remission [21]. Also, Arin et al. showed that rituximab injection was well tolerated and all patients showed good responses [22,23].

Penate et al. used intralesional rituximab for patients with primary cutaneous B-cell lymphoma. They showed that intralesional injection of rituximab, three times a week could result in a complete cure for 71% of

Table 2
Size of lesions and VAS comparison before and after treatment with rituximab and triamcinolone.

		Before treatment (a)	One month after the first injection (b)	Five months after second injection (c)	p value
Mean of size of lesion (cm^2)	Rituximab	1.4	0.59	0.36	a vs b: < 0.001 a vs c: < 0.001 b vs c: 0.065
	Triamcinolone	1.56	0.48	0.32	a vs b: < 0.001 a vs c: < 0.001 b vs c: 0.16
Mean of VAS	Rituximab	3.5		9	< 0.001
	Triamcinolone	3.5		8.9	< 0.001

Table 3
Size of lesions, VAS, and EI before and after treatment.

		Before treatment	p value	One month after the first injection	p value	Five months after the second injection	p value
Mean of size of the lesion (cm^2)	Rituximab	1.4	0.19	0.59	0.28	0.36	0.71
	Triamcinolone	1.56		0.48		0.32	
Mean of VAS	Rituximab	3.5	1.00			9	1.00
	Triamcinolone	3.5				8.9	
Mean EI	Rituximab					0.78	0.68
	Triamcinolone					0.77	

Table 4
PVLSS before and after treatment.

		PVLSS after treatment				p value
		Nil	Mild	Moderate	Severe	
PVLSS before treatment	Nil	1	0	0	0	0.17
	Mild	0	6	0	0	
	Moderate	1	2	2	0	
	Severe	2	2	2	1	

the patients [24]. Vinay et al. treated 3 resistant patients with PV using intralesional rituximab, at two doses of 5 mg/cm2, and all of the patients achieved clinical remission [25].

Intralesional Triamcinolone Acetonide (TA) is commonly used for treatment of oral PV lesions. Different studies showed an excellent response to triamcinolone therapy in patients with PV. Also, Mignogna et al. showed that, TA injection in oral PV results in complete clinical remission of lesions [26]. Also in a case report, Kozeis et al. showed that the injection of TA in a 76-year-old woman with severe PV lesions, caused complete remission after 1 year follow-up [27].

In the current study, for the first time, the effect of intralesional injection of triamcinolone was compared versus rituximab. To our best knowledge, this is the first randomized clinical trial study evaluated the efficacy of rituximab.

The results showed that PV lesion remitted after treatment with triamcinolone, and no relapse was observed in patients.

The results showed no statistically significant difference between the size of lesions, VAS, and EI after treatment with rituximab compared to triamcinolone. Regarding the results of the study, using rituximab or triamcinolone is recommended for treatment of the refractory oral or scalp lesions. It seems valuable to consider the use of intralesional rituximab or triamcinolone for treatment of a few refractory lesions of PV instead of using more aggressive systemic treatments. More studies with larger sample size and longer follow-up are suggested to better evaluate the efficacy of rituximab.

Acknowledgments

The authors thank all of the individuals who helped to perform this study.

References

[1] Y.H. Huang, C.F. Kuo, Y.H. Chen, Y.W. Yan, Incidence, mortality and causes of death of patients with pemphigus in Taiwan, J. Investig. Dermatol. 132 (1) (2012) 92–97.
[2] C. Chams-Davatchi, M. Valikhani, M. Daneshpazhooh, N. Esmaili, K. Balighi, Z. Hallaji, et al., Pemphigus: analysis of 1209 cases, Int. J. Dermatol. 44 (6) (2005) 470–476.
[3] H. Handa, P. Khare, V. Sharva, A Comprehensive Review on Pemphigus Vulgaris, (2019).
[4] E. Beutner, R. Jordon, Demonstration of skin antibodies in sera of pemphigus vulgaris patients by indirect immunofluorescent staining, Proc. Soc. Exp. Biol. Med. 117 (2) (1964) 505–510.
[5] A.R. Ahmed, J. Graham, R.E. Jordon, T.T. Provost, Pemphigus: current concepts, Ann. Intern. Med. 92 (3) (1980) 396–405.
[6] M. Kasperkiewicz, C.T. Ellebrecht, H. Takahashi, J. Yamagami, D. Zillikens, A.S. Payne, et al., Pemphigus, Nat. Rev. Dis. Primers. 3 (2017) 17026.
[7] W.F. Lever, G. Schaumburg-Lever, Immunosuppressants and prednisone in pemphigus vulgaris: therapeutic results obtained in 63 patients between 1961 and 1975, Arch. Dermatol. 113 (9) (1977) 1236–1241.
[8] S.W. Yeh, N. Sami, R.A. Ahmed, Treatment of pemphigus vulgaris, Am. J. Clin. Dermatol. 6 (5) (2005) 327–342.
[9] S. Mishra, A. Rizvi, A Review on Pemphigus Vulgaris: Rare but a Severe Auto Immune Disorder, (2013).
[10] A.R. Ahmed, Z. Spigelman, L.A. Cavacini, M.R. Posner, Treatment of pemphigus vulgaris with rituximab and intravenous immune globulin, N. Engl. J. Med. 355 (17) (2006) 1772.
[11] V. Prajapati, P. Mydlarski, Advances in pemphigus therapy, Skin Therapy Lett. 13 (3) (2008) 4–7.
[12] F. Jowkar, M. Sadat Sadati, S. Tavana, M.A. Agah, Epidemiology of autoimmune bullous diseases and therapeutic modalities during a 10 year period in Iran, Acta Dermatovenerol. Croat. 22 (4) (2014) 246.
[13] A. Baican, R. Chiorean, D.C. Leucuta, C. Baican, S. Danescu, D. Ciuce, et al., Prediction of survival for patients with pemphigus vulgaris and pemphigus foliaceus: a retrospective cohort study, Orphanet J. Rare Dis. 10 (1) (2015) 48.
[14] F. Iraji, A. Yoosefi, Healing effect of Pilocarpine gel 4% on skin lesions of pemphigus vulgaris, Int. J. Dermatol. 45 (6) (2006) 743–746.
[15] S. Grover, Scoring systems in pemphigus, Indian J. Dermatol. 56 (2) (2011) 145.
[16] Y. Teng, T. Huizinga, J. Van Laar, Targeted therapies in rheumatoid arthritis: focus on rituximab, Biologics Targets Ther. 1 (4) (2007) 325.
[17] K.A. Keogh, M.E. Wylam, J.H. Stone, U. Specks, Induction of remission by B lymphocyte depletion in eleven patients with refractory antineutrophil cytoplasmic antibody–associated vasculitis, Arthritis Rheum. 52 (1) (2005) 262–268.
[18] T.D. Levine, Rituximab in the treatment of dermatomyositis: an open-label pilot study, Arthritis Rheum. 52 (2) (2005) 601–607.
[19] T. Vallerskog, I. Gunnarsson, M. Widhe, A. Risselada, L. Klareskog, R. van Vollenhoven, et al., Treatment with rituximab affects both the cellular and the humoral arm of the immune system in patients with SLE, Clin. Immunol. 122 (1) (2007) 62–74.
[20] J.M. Meijer, P. Meiners, A. Vissink, F. Spijkervet, W. Abdulahad, N. Kamminga, et al., Effectiveness of rituximab treatment in primary Sjögren's syndrome: a randomized, double-blind, placebo-controlled trial, Arthritis Rheum. 62 (4) (2010) 960–968.
[21] E. Schmidt, S. Herzog, E.B. Bröcker, D. Zillikens, M. Goebeler, Long-standing remission of recalcitrant juvenile pemphigus vulgaris after adjuvant therapy with

rituximab, Br. J. Dermatol. 153 (2) (2005) 449–451.
[22] M. Arin, A. Engert, T. Krieg, N. Hunzelmann, Anti-CD20 monoclonal antibody (rituximab) in the treatment of pemphigus, Br. J. Dermatol. 153 (3) (2005) 620–625.
[23] L. Virgolini, V. Marzocchi, Anti-CD20 monoclonal antibody (rituximab) in the treatment of autoimmune diseases. Successful result in refractory pemphigus vulgaris: report of a case, Haematologica 88 (7) (2003) (ELT24-ELT).
[24] Y. Peñate, B. Hernandez-Machin, L. Perez-Mendez, F. Santiago, B. Rosales, O. Servitje, et al., Intralesional rituximab in the treatment of indolent primary cutaneous B-cell lymphomas: an epidemiological observational multicentre study. The Spanish Working Group on Cutaneous Lymphoma, Br. J. Dermatol. 167 (1) (2012) 174–179.
[25] K. Vinay, A.J. Kanwar, A. Mittal, S. Dogra, R.W. Minz, T. Hashimoto, Intralesional rituximab in the treatment of refractory oral pemphigus vulgaris, JAMA Dermatol. 151 (8) (2015) 878–882.
[26] M. Mignogna, G. Fortuna, S. Leuci, D. Adamo, G. Dell'Aversana Orabona, E. Ruoppo, Adjuvant triamcinolone acetonide injections in oro-pharyngeal pemphigus vulgaris, J. Eur. Acad. Dermatol. Venereol. 24 (10) (2010) 1157–1165.
[27] N. Kozeis, S. Tyradellis, E. Dragiotis, H. Eleftheriadis, Triamcinolone acetonide for rare ocular manifestations of pemphigus vulgaris: a case report, Clin. Ophthalmol. 4 (2010) 365 (Auckland, NZ).