Available online at www.sciencedirect.com
ScienceDirect
Transfusion and Apheresis Science 39 (2008) 109–113
TRANSFUSION AND APHERESIS SCIENCE
www.elsevier.com/locate/transci

Immunoadsorption method using immunoglobulin Adsopak in adult cases with ITP resistant to splenectomy and other medical therapies

Oktay Bilgir [a,*], Ferda Bilgir [b], Mehmet Calan [a], Levent Kebapcilar [a], Esin Kulac [c]

[a] *Izmir Education and Research Hospital, 2nd Clinic of Internal Medicine, 35380 Izmir, Turkey*
[b] *Buca State Hospital, Department of Internal Medicine, Izmir, Turkey*
[c] *Department of Health Service, Kastamonu, Turkey*

Abstract

Background: Despite all medical therapies and splenectomy performed, severe life-threatening thrombocytopenia persists in many adult patients.

Aim of the study: To evaluate the efficiency of Immunoglobulin Adsopak immunoadsorption method in eliminating circulating immunocomplexes and IgG from plasma in these cases.

Methods: The method of POCARD Ig-Adsopak Wide Spectrum was applied to four cases with severe thrombocytopenia ($\leqslant$20.000/μL) by treating with a daily plasma of 0.25–2 L for 6 days. Platelet counts were recorded at the beginning and during administration of the method. For long-term effects, the cases were called for monthly controls during which platelet counts were measured.

Results: Platelet counts of four cases started to increase on average as of the first day. On Day 11, the platelet count reached 50.000/μL. On the following days (Day 17), platelet count was recorded as 90.000/μL. Cases were followed up for a mean of 2 years. At the end of 2 years, mean platelet count of the patients was 210.000 (175.000–265.000) μL.

Conclusion: In adult cases who do not respond to surgical and medical therapy, including splenectomy, the results obtained from the therapy using the method of Immunoadsorption with Immunoglobulin Adsopak are promising both in short and long-term follow up.

Keywords: Treatment resistant ITP; Immunoadsorption therapy

1. Introduction

Idiopathic thrombocytopenic purpura (ITP) is an acquired, autoimmune hemorrhagic disease characterized with resistant thrombocytopenia, bleeding episodes and occasionally an elevation of megakaryocytes in bone marrow but often showing a normal bone marrow. In ITP, anti-platelet antibodies and circulating immunocomplexes lead to the elimination of the platelets in spleen and RES [1].

For the treatment of ITP, especially glucocorticoids as well as splenectomy, vinka alkaloids,

* Corresponding author. Tel.: +90 232 2505050x1276; fax: +90 232 2614444.
E-mail address: obilgir2001@yahoo.com (O. Bilgir).

1473-0502/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.transci.2008.07.006

danazol, colchicine, ascorbic acid, cyclophosphamide, azathiopurine and high-dose intravenous gammaglobuline (IV IgG) are administreted [2]. In addition to these treatments, in cases with ITP, the immunoadsorption therapy which allows the therapeutic removal of IgG and immunocomplexes containing IgG from the patient's plasma was introduced [3]. In the subsequent studies, the long-term follow-up of immunoadsorption therapy was demonstrated [4]. In this study, we aimed to show that the administration of immunoadsorption therapy could be a life saving method in adult cases with chronic ITP resistant to all types of medical treatment, including splenectomy, in short and long term.

2. Material and methods

2.1. Patients

Four cases with chronic ITP (four female patients, mean age, 34, 25) and previously administreted treatments are showed in Table 1. Before the administration of this therapy method, the cases were followed up on average for forty months. Despite the previously administreted treatments, the mean platelet count of the patients was 8.000/μL. Patients had ecchymotic lesions on the extremities. Two patients were presented with menometrorhagy. The therapies indicated in the tables were administreted to the patients at different times; however, an immunoadsorption therapy was planned since the patient was considered unresponsive due to platelet counts that didn't show any increase during the subsequent controls.

2.2. Treatment procedure

Before administrating this treatment modality to patients, blood pressure and pulse was checked and a physical examination was performed. These controls were performed after each procedure. Patients were started on a standard dose of 32 mg/day of methylprednisolon. Method was administrered every other day and for 6 times by using IgG-Adsopak which contained sheep antibodies. Once the platelet count reached 50.000/μL, treatment was stopped and patients were just followed up. We performed a cut down all patient because we had not found large venos vessel for the technique. During the first 3 days, mean 2 Units of platelet transfusion were given. Because of bleeding area of cut down. During the application of this procedure, proximal vein of upper extremity of patients were used. During the procedure, 1/12 ACD solution was given. This process was centrifuged by using Dideco Excel Separator. Mean duration of process was 235 min.

The adsorption itself is done in a pair of immunoadsorption columns Ig-ADSOPAK® (POCARD Ltd., Moscow, Russia). The column consists of a glass container with adsorbent that is an inert carrier of Sepharosa Cl-4B with covalently bound polyclonal sheep antibodies against human immunoglobulins. The column adsorption capacity is 6–8 mg of immunoglobulins/ml of adsorbent, the column volume is 200 ml.

Before the first use, adsorption columns were thoroughly rinsed with 1000 ml of physiologic solution (flow rate 140 ml/min), 1000 ml of glycine buffer (flow rate 75 ml/min), 1000 ml of PBS buffer (flow rate 140 ml/min), and again with 1000 ml of physiologic solution (flow rate 140 ml/min). Prepared columns were then rinsed with 1000 ml of preserving solution PBS buffer with 0.01% of NaN_3 (Medicap Clinic GmbH, Ulrichstein, Germany) and stored at a temperature of 2–8 °C. One pair of adsorption columns is for one patient only.

2.3. Laboratory tests

Before performing this procedure, a cut down was performed in the patients. Before and after each immunoadsorption therapy, routine hemogram (WBC, Hemoglobin, hematocrit and platelet) and biochemical tests (urea, creatinine, SGOT, SGPT, GGT, Blood glucose) were performed and IgG, A and M levels were measured for each patient. Coul-

Table 1
Patients and the previous treatments administered

Patients	Gender	Age	Steroid	IVIG	Splenectomy	Other treatments
Patient 1	Female	40	+	+	+	Azathiopurine
Patient 2	Female	28	+	+	+	Azathiopurine
Patient 3	Female	66	+	+	+	Cyclophosphamide
Patient 4	Female	23	+	+	+	Cyclophosphamide

ter Gene-S analyzer (Beckman Coulter, USA) for blood count and ILAB-1800 photometric method for biochemical tests were used.

Routine examinations of concentrations of plasma complement constituents C3 and C4 were done before and after treatment procedure by a nephelometric method using İmage immun chemistry (Beckman Coulter, USA) in cooperation with the Institute of Clinical Biochemistry, Izmir Education Hospital Concentrations of C3:79–152 mg/dL and C4:16–38 mg/dL were considered normal.

Table 2
The baseline and on-therapy platelet counts

Days	Patient 1(/μL)	Patient 2 (/μL)	Patient 3 (/μL)	Patient 4 (/μL)
Baseline	8.000	10.000	10.000	4.000
Day 1	13.000	13.000	16.000	8.000
Day 3	17.000	20.000	20.000	23.000
Day 5	18.000	20.000	25.000	43.000
Day 7	28.000	27.000	28.000	53.000
Day 9	37.000	35.000	36.000	65.000
Day 11	50.000	50.000	44.000	80.000
Day 13	60.000	55.000	55.000	100.000
Day 15	65.000	70.000	60.000	120.000
Day 17	70.000	90.000	75.000	127.000

Table 3
Mean and standard deviation values for platelet counts

Platelet counts	*N*	Mean	Standard deviation	Minimum	Maximum
Baseline	4	8000.00	2828.427	4000	10,000
Day 7	4	34000.00	12675.436	27000	53000
Day 17	4	90500.00	25774.665	70000	127000

Test statistics: mean platelet counts at the beginning, at Day 7 and at Day 17 showed a significant statistical difference. Friedman test. *p*: 0.018(a).

2.4. Statistical analysis

Data were analyzed with Friedman test. All results were calculated as Mean ± SD. Baseline values, values obtained during and at the end of the procedure were compared. P value was found to be significant (*p*: 0.018).

3. Results

The results are shown in Tables 2–4. Pre- and post-procedure platelet counts are tabulated in Table 2 and immunoglobulin levels in Table 4. Mean platelet count reached 50.000/μL beginning from Day 11, and after the subsequent 2-month follow-up, mean platelet count reached 160.000 (145.000–178.000) μL. In addition, in terms of immunoglobulin levels, post-treatment IgA level showed a decrease of 25%, IgG levels showed a decrease of 30% and IgM levels showed a decrease of 35% compared to pre-treatment. In terms of C3, C4 levels, post-treatment C3 level showed a decrease of 32.2%, C4 level showed a decrease of 21.2% compared to pre-treatment (Tables 5 and 6).

Table 4
Mean and standard deviation for pre- and post-therapy immunoglobulin levels

	N		Mean	Standard deviation	Minimum	Maximum
IgA	4	Pre-therapy	416.25	20.15	395.00	440.00
		Post-therapy	315.50	67.79	218.00	368.00
IgG	4	Pre-therapy	1377.25	89.08	1250.00	1452.00
		Post-therapy	965.00	86.56	899.00	1030.00
IgM	4	Pre-therapy	277.50	35.97	198.00	278.00
		Post-therapy	182.25	13.12	166.00	196.00

Table 5
Mean and standard deviation values for processed plasma

	N	Range	Minimum	Maximum	Mean	Standard deviation
Seans 1	4	800.00	4200.00	5000.00	4650.00	341.56
Seans 2	4	600.00	4200.00	4800.00	4550.00	300.00
Seans 3	4	900.00	4100.00	5000.00	4525.00	377.49
Seans 4	4	800.00	4200.00	5000.00	4600.00	326.59
Seans 5	4	800.00	4000.00	4800.00	4550.00	378.59
Seans 6	4	800.00	4000.00	48000.00	4500.00	346.41

Table 6
Mean and standard deviation for pre- and post-therapy C3 and C4 levels

	N		Mean	Standard deviation	Minimum	Maximum
C3	4	Pre-therapy	183.50	16.01	160.00	195.00
		Post-therapy	126.25	10.90	112.00	138.00
C4	4	Pre-therapy	34.75	6.80	28.00	44.00
		Post-therapy	27.75	6.65	20.00	36.00

4. Discussion

As is known, cases with chronic treatment-resistant ITP still represents one of the most important problems encountered by hematologists, despite the use of new treatment modalities in view of the recent advances. In this group of patients, a few patients with chronic ITP which do not respond to standard and megadose glucocorticoids, Danazol, Azathopurine, Cyclophosphamide, splenectomy are included. Recently, Rituximab and even Peripheral Stem Cell Transplantations were performed and reported to be beneficial for the patients. However, these treatment modalities have challenges such as high-cost and finding a compatible donor, as is the case in transplantation procedure [5–7].

Most antiplatelet autoantibodies are IgG; remainder are IgM and IgA. Antibody-coated platelets bind antigen presenting cells through Fcγ receptors, primarily in the spleen but also in other organs of the mononuclear phagocyte system. It has been postulated that platelet destruction also amplifies the immune response. The mechanism involves presentation of platelet antigens by activated antigen presenting cells, which thereby activate boht CD4+ T cell clones and antigen specificities, induce different B cell clones to produce antibodies against distinct platelet antigens [16,17].

Complement activation plays a role in thrombocytopenia in some patients with ITP. Increased platelet associated C3, C4 and C9 have been demonstrated on the platelets from patients with ITP. In vitro studies have shown that, in the presence of antiplatelet antibodies, C3 and C4 can bind platelets and cause their lysis [18–20].

Immunoadsorption method was previously investigated by various authors as well and it was highlighted that the majority of the patients showed remission. However, Synder et al. administered this treatment modality in periods of 3–4 weeks and reported that 46% of the cases responded [4]. Our method allowed achievement of a response in a short time with administration performed every other day and for a total of 6 occasions. However, this response was not short-lived. In the cases followed up during a period which exceeded 2 years on average, a reversal in terms of platelet count was not observed. Leitner et al. published that they obtained a response only in one of the 3 cases with immunoabsorption method. In this treatment modality, very few side effects are observed. Transmission of infection via blood products which is the most frightening one is not observed [13–15]. In addition, immunoadsorption with polyclonal sheep antibodies used against human immunoglobulin leads to a substantial decrease in immunoglobulin levels [10–13]. In conclusion, this immunoadsorption method used is a treatment option with both high efficacy and very few side effects.

References

[1] Bussel JB. Autoimmune thrombocytopenic purpura. Hematol Oncol Clin North Am 1990;4:179.
[2] Berchtold P, McMillan R. Therapy of chronic idiopathic thrombocytopenic purpura in adults. Blood 1989;74: 2309.
[3] Pinsky CM. Selective removal of plasma components to achieve immune modulation. Semin Hematol 1989;26:1.
[4] Snyder HW, Cochran SK, Balint JP, et al. Experience with protein A immunoadsorption in treatment resistant adult immune thrombocytopenic Purpura. Blood 1992;79: 2237–45.
[5] Schiavotto C, Castaman G, Rodeghiero F. Treatment of ITP in patients with refractoriness to or with contraindication for corticosteroids and/or splenectomy with immunosuppressive therapy and danazol. Haematologica 1993; 78(6 Suppl. 2):29–34.
[6] Pamuk GE, Pamuk ON, Baslar Z, et al. Overview of 321 patients with ITP. Retrospective analysis of the clinical features and response to therapy. Ann Hematol 2002;81(8): 436–40.
[7] Braendstrup P, Bjerrum OW, Nielsen OJ, et al. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adult refractory idiopathic thrombocytopenic purpura. Am J Hematol 2005;78(4):275–80.
[10] Ptak J. Changes of plasma proteins after immunoadsorbtion using Ig-Adsopak columns in patients with myasthenia gravis. Transfusion Apheresis Sci 2004;30:125–9.

[11] Ptak J. Changes of selected hematologic parameters during long term immunoadsorption therapy. Transfusion Apheresis Sci 2005;33:299–304.
[12] Braun N, Bosch T. İmmunoadsorption,current status and future developments. Expert Opin Inv Drug 2000;9:2017–37.
[13] Blaha V, Solichova D, Zadak Z, Blaha M, Havel E, Vyroubal P, et al. Immunoadsorption in the treatment of hypercholesterolemia: five years of research and clinical experience. Nutrition 2002;12:211.
[14] Blaha M, Zadak Z, Blaha V, Havel E, Vyroubal P, Pivokonska M, et al. LDL-apheresis as a method of extracorporal LDL- elimination. Ateroskleroza 2001;5: 19–23.
[15] Leitner GC, Stiegler G, Horvath M, Hoecker P, Sagaster P, Panzer S. Idiopathic autoimmune thrombocytopenia: Evidence for redistribution of platelet antibodies into the circulation after immunoadsorption treatment. Am J Hematol 73(1):44–7.
[16] He R, Reid DM, Jones CE, Shulman NR. Spectrum of Ig classes specificities and titers of serum antiglycoprotenis in chronic idiopathic thrombocytopenic purpura. Blood 1994;83:1024.
[17] Cines DB, Blanchette VS. İmmune thrombocytopenic purpura. N Engl J Med 2002;346:995.
[18] Hauch TW, Rosse WF. Platelet bound complement in immune thrombocytopenia. Blood 1997;50:1129.
[19] Kurata Y, Curd JG, Tamerius JD, Mc Millan R. Platelet associated component in chronic I.T.P.. J Haematol 1985;60: 723.
[20] Tsubakio T, Tani P, Curd JG, McMillan R. Complement activation in vitro by antiplatelet antibodies in chronic immune thrombocytopenic purpura. Br J Haematol 1986;63:293.