


ORIGINAL ARTICLE

Profile of the single-use, multiple-pass protein A adsorber column in immunoadsorption

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Abstract

Background and Objectives: Immunoadsorptions (IA) are used to remove autoantibodies from the plasma in autoimmune disorders. In this study, we evaluated the effects of a single-use, recombinant staphylococcal protein A-based immunoadsorber on blood composition of the patient.

Materials and Methods: In a cohort of patients with myasthenia gravis or stiff-person syndrome, essential parameters of blood cell count, coagulation, clinical chemistry or plasma proteins and immunoglobulins (Ig) were measured before and after IA ($n = 11$).

Results: In average, IA reduced the levels of total IgG, IgG1, IgG2 and IgG4 by approximately 60%, the acetylcholine receptor autoantibody levels by more than 70%. IgG3, IgA or IgM were diminished to a lower extent. In contrast to fibrinogen or other coagulation factors, the column markedly removed vitamin K-dependent coagulation factors II, VII, IX and X by approximately 40%–70%. Accordingly, international normalized ratio and activated partial thromboplastin time were increased after IA by 59.1% and 32.7%, respectively. Coagulation tests almost returned to baseline values within 24 h. Blood cell count, electrolytes, total protein or albumin were not essentially affected. No clinical events occurred.

Conclusion: The single-use, multiple-pass protein A adsorber column is highly efficient to remove IgG1, IgG2 and IgG4 or specific acetylcholine receptor autoantibodies from the plasma. Coagulation parameters should be monitored, since the column has the capacity to largely reduce vitamin K-dependent factors.

KEYWORDS

apheresis technologies, apheresis-therapeutic, blood processing, haemostasis, plasma

INTRODUCTION

The removal of autoantibodies from plasma is an option for the treatment of patients with severe autoimmune-mediated diseases [1]. The

depletion of autoantibodies is associated with clinical benefits, for example, in several neurological disorders like crisis of myasthenia gravis caused by acetylcholine receptor antibodies or stiff-person-syndrome triggered by glutamic acid decarboxylase antibodies [2–5].

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For that purpose, therapeutic plasma exchanges (TPE) or immunoadsorptions (IA) can be used as technical procedures [1, 3]. In both, plasma separation can be performed by membrane filtration or centrifugation. In TPE, plasma is unselectively wasted and substituted by albumin, electrolyte solutions or fresh-frozen plasma. In IA, plasma is redirected to the patient after passing an adsorber column for the elimination of autoantibodies without the need of additional fluid substitution. A number of adsorber columns have been developed for clinical use, characterized by different affinities and efficacies [6]. Immune adsorber columns can be designed for single-use or for regenerative use, designed as single-column or a double-column system. Recently, a single-use, multiple-pass immunoadsorber based on recombinant staphylococcal protein A as ligand became available for clinical practice [7].

For the estimation of potential adverse effects, it is important to analyse the impact of the novel column on blood composition. The aim of this study was to investigate the performance of IA with the column focusing on blood count, coagulation, plasma proteins and clinical chemistry parameters before and after the procedures in a cohort of patients with myasthenia gravis or stiff-person-syndrome.

MATERIALS AND METHODS

In total, 16 IA procedures were performed in seven patients with autoimmune-mediated neurological disorders (six patients with myasthenia gravis, one patient with stiff-person-syndrome) during their treatment series. The preanalytical requirements for the projected laboratory investigations were met in 11 IA procedures, which were included in the study. Detailed characteristics of patients are listed in Table 1.

The study was performed according to the Declaration of Helsinki and approved by the local ethics committee of the university of Wuerzburg (approval number 256/12). All participants provided their written informed consent.

Contraindications for IA according to the manufacturer's instructions like severe cardiovascular diseases or acute systemic infections were ruled out. The patients had no history of bleeding episodes, no current surgical interventions or fresh wounds. Platelet counts were within the normal range.

IA were performed with the single-use, regenerative, multiple-pass protein A adsorber column (Ligasorb, Fresenius Medical Care AG & Co. KGaA, Bad Homburg, Germany) connected with the ADA-sorb device (Medicap Clinic GmbH, Ulrichstein, Germany) following the manufacturers' instructions. In this column, the ligand, recombinant staphylococcal protein A, is covalently bound to the matrix consisting of polymethacrylate beads. During the procedure, the column is initially loaded with plasma enabling the attachment of immunoglobulins (Ig) to protein A. Processed plasma is redirected to the cell separator and finally back to the patient. In a consecutive regeneration step, plasma is displaced and immunoglobulins are eluted from the column. Fluids from elution are discarded. These cycles are

performed repeatedly, automatically controlled by the ADA-sorb device, allowing the treatment of several litres plasma volume [7].

Plasma was generated by the cell separator Spectra Optia (Terumo BCT, Lakewood, CO). Plasma volume was calculated using nomograms considering height, weight and haematocrit [8]. As target of the IA, approximately one and a half of the calculated patient's plasma volume was processed by the column and redirected to the patient. The ratio of anticoagulant (anticoagulant citrate dextrose solution A) to inlet blood volume was 1:12.

Directly before and 15 min after finalization of the IA, blood samples were drawn from a peripheral vein for the analysis of blood composition. The samples were collected in appropriate tubes (Sarstedt, Nuembrecht, Germany) containing 3.2% citrate buffer (106 mM trisodium citrate) for the analysis of coagulation parameters or containing 1.6 mg/ml ethylenediaminetetraacetic acid for blood cell count. Tubes without anticoagulant served for the analysis of plasma proteins and immunoglobulins. The tubes were directly sent to the laboratory for further processing.

Blood cell count was performed with the haematology analyser Sysmex KX-21 N (Sysmex Europe GmbH, Norderstedt, Germany). Coagulation parameters were determined on a BCS XP analyser (Siemens Healthcare Diagnostics, Marburg, Germany), clinical chemistry and plasma proteins on Roche cobas analysers (Roche Diagnostics GmbH, Mannheim, Germany) using appropriate reagents. Acetylcholine receptor autoantibodies were measured by a specific radioimmunoassay.

Statistical analysis

Descriptive and statistical data were calculated with the GraphPad PRISM 7 programme (GraphPad Software, San Diego, CA). Data distribution analysis was performed with the Shapiro-Wilk test. Differences of variances between groups were analysed by one-way analysis of variance or by paired Student's *t*-test as appropriate. $p < 0.01$ was considered statistically significant.

RESULTS

Performance of IA with the single-use, multiple-pass protein A adsorber

The mean duration of the IAs was 295 ± 35 min resulting in the mean processed volume of $183 \pm 24.7\%$ in relation to the patients' plasma volume (Table 2). The administered citrate solution was 1038 ± 132 ml. Adverse clinical events did not occur, the procedures were well tolerated by the patients.

Before hospital stay, the disease-specific symptoms had increased in all patients despite drug therapy providing the indication for apheresis treatment to alleviate immobility and severe pain or to avoid mechanical ventilation (in the case of myasthenia gravis). The patients

TABLE 1 Characteristics of treated patients

Patient	Age (years)/gender	Body weight (kg)	Estimated plasma volume (ml)	Diagnosis	Auto-antibodies	Main symptoms	Specific drugs	Time (days)	Series of treatments
1	22/female	53	2100	Myasthenia gravis	MuSK	Ocular impairment, dysphagia, muscle weakness	M, P	9	IA-IA-IA-TPE-TPE
2	60/female	80	3400	Stiff-person-syndrome	–	Muscle rigidity, cramps	B, O	4	IA-IA
3	31/female	60	2200	Myasthenia gravis	AChR	Ocular impairment, muscle weakness	A, P	7	IA-TPE-IA
4	77/male	79	3200	Myasthenia gravis	AChR	Ocular impairment, dysphagia, muscle weakness	A, P, S	3	IA-IA
5	51/male	79	3400	Myasthenia gravis	AChR	Muscle weakness, ocular impairment	M, P	3	IA-IA-TPE
6	50/female	60	2700	Myasthenia gravis	AChR	Dysarthria, dysphagia	M, P, S	9	IA-TPE-IA-TPE-TPE
7	72/male	92	4000	Myasthenia gravis	AChR	Dysarthria, dysphagia	C, P	17	IA-IA-TPE-IA

Note: The patients received a series of apheresis treatments, in individual combinations of IA and TPE. IA procedures indicated by bold letters were included into the evaluation (n = 11). Other IA were not included in the study, since it was not possible to ascertain the preanalytical requirements for laboratory analysis at every time.

Abbreviations: A, azathioprine; AChR, acetylcholine receptor; B, benzodiazepine; C, cyclosporin; IA, immunoadsorption; M, mycophenolate mofetil; MuSK, muscle-specific kinase; O, opioid; P, pyridostigmine; S, steroid; time, time from starting apheresis treatment until discharge from hospital; TPE, therapeutic plasma exchange.

TABLE 2 Details on performed immunoadsorption

Number of procedures	11
Processed volume (ml) (mean ± SD)	5486 ± 744
Processed volume/plasma volume (%) (mean ± SD)	183 ± 24.7
Citrate volume (ml) (mean ± SD)	1038 ± 132
Mean duration (min) (mean ± SD)	295 ± 35.4
Vein access central/peripheral	0/11
Adverse events	None

substantially recovered from symptoms after completed series of treatments consisting of an individual combination of IA and TPE as depicted in Table 1. In the clinical course, none of the patients required mechanical ventilation. The time period from starting apheresis procedures until discharge from hospital was 3 to 17 days.

Immunoglobulins and proteins

In eight of the procedures with patients suffering from myasthenia gravis, measurable levels of acetylcholine receptor antibodies were detected before IA, with a wide range from 0.2 nmol/L to almost 30 nmol/L. In each individual, the IA led to a substantial reduction of the autoantibody level as depicted in Figure 1. In average, the level dropped by 70.3% (Table S1). The total amount of immunoglobulins was depleted by 58.9%. Regarding the immunoglobulin subclasses, IA diminished IgG, IgG1, IgG2 and IgG4 by approximately 60% or more (Figure 2). The reductive effect was less prominent for IgG3, IgA or IgM with approximately 20%–40%, and similarly weak for the complement factors 3 and 4 (Table S1).

The total protein concentration of plasma showed lower values after the procedure, for albumin by 14.7% and for total protein by 22%, presumably caused by the dilutive effect of administered citrate solutions with more than 1 L during the IA rather than column-dependent depletion (Table S1).

Blood cell count and clinical chemistry

Blood cell count remained stable during the IA, except for a small decrease of platelets from $216 \pm 40 \times 10^3/\mu\text{l}$ to $184 \pm 40 \times 10^3/\mu\text{l}$ (Table S2). Remarkably, the leukocyte levels remained unchanged. Parameters of clinical chemistry were not relevantly affected. The increase of total calcium from 2.3 ± 0.1 to 2.8 ± 0.2 mmol/L is explained by continuous administration of low-dose calcium during the IA, added into the line back to the patient.

Coagulation factors

The global coagulation tests showed distinct deviations after the procedures (Table S3). In average, values of international normalized ratio (INR) rose by 59.1%. Activated partial thromboplastin time (aPTT)

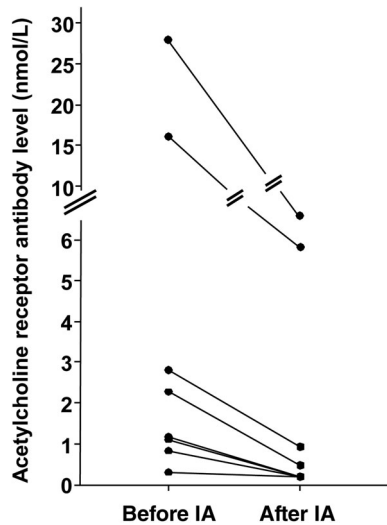


FIGURE 1 Reductive effect of immunoadsorption (IA) on acetylcholine receptor antibody levels. The individual autoantibody levels in patients with myasthenia gravis before and after IA are shown; $n = 8$

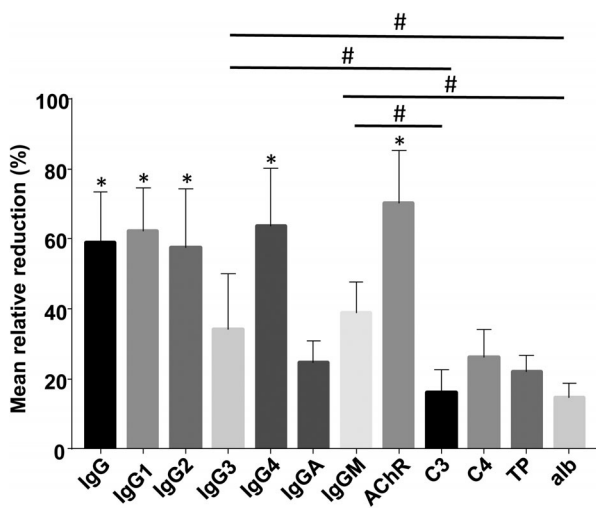


FIGURE 2 Depletion of plasma proteins and immunoglobulins by immunoadsorption (IA). The relative reduction after IA is shown for each parameter as mean \pm SD (in % related to the value before IA). * $p < 0.01$ (compared to IgG3, IgA, IgM, C3, C4, TP or alb); # $p < 0.01$ (compared as indicated); $n = 11$. AChR, acetylcholine receptor antibody; alb, albumin; C3, complement factor 3; C4, complement factor 4; Ig, immunoglobulin; TP, total protein

developed a prolongation from 34.7 ± 5.8 s to 45.8 ± 8.2 s. Fibrinogen (factor I) was only slightly reduced by approximately 25% from 2.24 ± 0.35 g/L to 1.71 ± 0.49 g/L, comparable to the coagulation factors V, VIII, XI, XII, XIII or to the von Willebrand parameters (Table S3). In contrast, the vitamin K-dependent coagulation factors showed a pronounced reduction, with factor II by almost 67%, factor VII by 51%, factor IX by 42% and factor X by 66% as outlined in Figure 3. The d-dimer levels were generally very low with stable values after the IA.

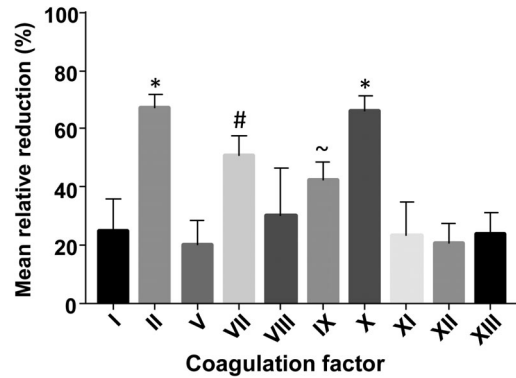


FIGURE 3 Reduction of coagulation factors by immunoadsorption (IA). The relative reduction after IA is shown for different coagulation factors as mean \pm SD (in % related to the value before IA). * $p < 0.01$ (compared to all parameters with the exception of factors II and X); # $p < 0.01$ (compared to all parameters with the exception of factors II, VII and X); ~ $p < 0.01$ (compared to all parameters with the exception of factors II, VII, IX and X); $n = 11$

In six patients, the following IA was performed the next day (16–20 h after the initial IA). In these cases, before starting IA, the analysis of blood coagulation revealed an increase of factors II, VII, IX and X to values within or close to the reference range (Figure S1). In addition, INR values recovered from 1.65 ± 0.13 to 1.16 ± 0.10 . aPTT returned to remarkably lower values from 43.8 ± 4.9 s to 34.9 ± 6.0 s in average. According to liver enzymes and to liver synthesis parameters, the six patients had no impairment of liver function. The increase of fibrinogen was less emphasized from 1.75 ± 0.56 g/L to 2.20 ± 0.50 g/L.

DISCUSSION

In accordance to a previous report by Sufke et al. [7], the single-use, multiple-pass protein A adsorber column has proved to be highly efficient to remove immunoglobulins of type IgG1, IgG2 and IgG4 from the plasma, with a reduction of approximately 60% after performance of a single IA. The depletion rate for specific acetylcholine receptor autoantibodies was more than 70% and is comparable to the characteristics of the tryptophan immune adsorber [9, 10].

There were no adverse clinical effects associated with the performed IA and no technical problems occurred. The number of procedures provided sufficient and consistent data on effects related to blood composition. However, further trials with larger patient cohorts are required to obtain profound evidence for the safety profile of the column.

The adsorber as a single-use device is favourable to patients requiring only few treatment sessions without need of recurrent therapy due to lower costs. In contrast to the tryptophan immune adsorber with limited loading volumes [11], the single-use, multiple-pass protein A adsorber column is technically able to process large amounts of plasma. However, the intermittent regeneration cycles of the single column contribute to time-consuming procedures. The long

duration of IA with almost 6 h should be considered as a disadvantage and could, for example, raise problems with peripheral vein access.

Regarding the influence on blood composition, the blood cell count or the electrolyte levels were not essentially tampered by the IA. The slightly reduced levels of albumin or other plasma proteins by 10%–20% may be interpreted as a dilutive effect, since a large volume of citrate solution (with more than 1 L) was administered during the procedures. Benny et al. showed that IA using protein A agarose columns do not relevantly remove albumin [12], similarly to IA with tryptophan immune adsorber column [13].

Since anticoagulation is required for extracorporeal circuits, the occurrence of bleeding complications is a major concern [14]. In TPE, the removal of coagulation factors is a limitation of treatment frequencies or exchanged plasma quantities unless wasted plasma is at least partially substituted by fresh-frozen plasma [15, 16]. In this context, the redirection of the patient's plasma from the column without need for substitution is an important advantage of IA.

Although efficacious for the removal of autoantibodies, IA with tryptophan immune adsorber were associated with the strong depletion of fibrinogen [11, 17]. In this case, fibrinogen obviously exerts strong hydrophobic and electrostatic interactions with the tryptophan's bulky indole ring [18]. The single-use, multiple-pass protein A adsorber column has only weak effects on fibrinogen levels, with an average reduction of 25%, which is most likely caused by dilution and in line with the recent study by Sufke et al. [7]. A low impact on fibrinogen levels have also been reported for other adsorbers using the ligand protein A from *Staphylococcus aureus*, sheep antibodies against human IgG or the specific oligopeptide Gam 146 [19].

However, IA with the investigated column led to deviations of the global coagulation tests, INR and aPTT. The analysis of single factors revealed that the depletion of vitamin K-dependent coagulation factors II, VII, IX and X by approximately 40%–70% is responsible for that phenomenon. In contrast, other coagulation factors were only diminished by 20%, compatible with the dilutive effect as described.

The molecular mechanisms in the column contributing to the reduction of vitamin K-dependent coagulation factors was not subject of this study and deserves further experimental investigations. For example, the depletion could refer to γ -carboxyglutamic acid residues as a typical characteristic of these proteins [20], but the adherence to surfaces is dependent on complex interactions between plasma proteins and the column matrix. Interestingly, the use of an adsorber with polymethacrylate-bound albumin for endotoxin elimination led to prolongation of aPTT [21]. These processes could be influenced by electrostatic, hydrophobic characteristics of the protein or mediated by specific-site binding. In addition, the nature of protein A, in this case as a recombinant protein, may have an influence on binding characteristics.

The kind of anticoagulant or chemical properties of the plasma like the pH value or the calcium ion concentration may also interfere with binding [22]. In this regard, it would also be of interest to examine protein C and S, as further vitamin K-dependent coagulation factors.

As a practical approach in patient care, the global coagulation tests should be monitored during IA therapies with single-use, multiple-pass protein A adsorber, especially before invasive diagnostic or therapeutic procedures. In patients without liver dysfunction, the recovery of the coagulation system may be expected within 24 h since the values of vitamin K-dependent factors, aPTT and INR almost returned to baseline values in this time span.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

N.S. contributed to the research design, acquisition of data, analysis and interpretation of data. G.V., J.G., J.V., M.N. and P.K. contributed to the acquisition of data. N.Ü. and E.K. contributed to the drafting and revising the paper. A.K. contributed to the analysis and interpretation of data. M.B. contributed to the research design, drafting and revising the paper. J.K. contributed to the research design, analysis and interpretation of data, drafting and revising the paper.

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SUPPORTING INFORMATION

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