TECHNICAL PERFORMANCE OF A NOVEL ADSORPTIVE TYPE CYTAPHERESIS MODULE IN PATIENTS WITH MODERATELY TO SEVERELY ACTIVE ULCERATIVE COLITIS

Grit Waitz[1], H. Prophet[2], Gisela Sparmann[1], J. Emmrich[3], W. Ramlow[2]

[1]BioArtProducts GmbH, Rostock , [2]Apheresis Center Rostock, [3]Division of Gastroenterology, University of Rostock, Germany

INTRODUCTION

Platelets have been recognized to play an important role in the pathophysiology of inflammatory bowel disease (IBD). Their number is increased during flare ups and correlates with disease severity. Platelets are able to activate various cells, e. g. through contact with CD40L, secrection of soluble CD40L and other chemokines mediating leukocyte adhesion and transmigration[1,2]. Therefore, activated platelets are potential therapeutic targets in IBD.

Granulocyte/monocyte adsorption apheresis (GMCAP) is an effective treatment option for patients with active ulcerative colitis (UC). It has been shown that the efficiency of the platelet reduction by leukocytapheresis was correlated with the therapeutic response[3,4].

Immunopure® is a novel adsorptive type cytapheresis module which particularly removes platelets, granulocytes and monocytes from the peripheral blood. A prospective open label pilot study was conducted to evaluate the biocompatibility, technical performance and changes in circulating platelets/leukocytes of this device in patients with active UC.

PATIENTS AND METHODS

Demographic data:

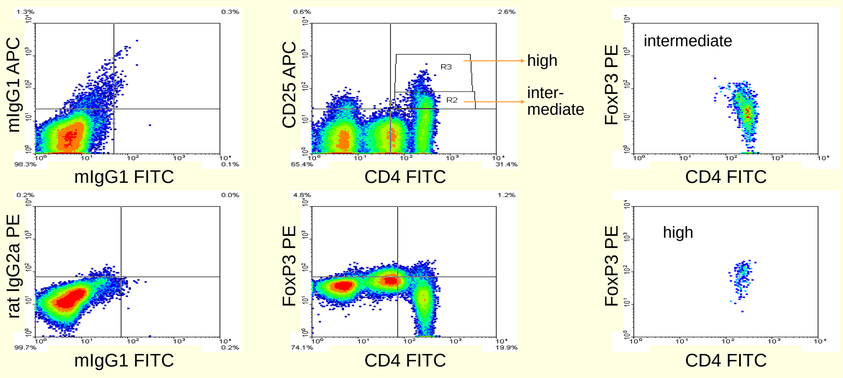
10 patients (6 male, 4 female, mean age: 47.1 years, minimum age: 25 years, maximum age: 73 years) with moderately to severely active UC, defined by Clinical Activity Index (CAI according to Rachmilewitz[5]: 6-10), who have failed to achieve long-term remission with steroids and/or immunosuppressants or who were contraindicated or intolerant to steroids and/or immunosuppressants were recruited.

The Immunopure® (Nikkiso, Japan) device has been specifically designed to be used in a simple hemoperfusion setting for the removal of activated granulocytes, monocytes and platelets. The device is a gamma-ray sterilized single use (disposable) module filled with amorphous polyarylate resin beads of 1.0 mm diameter. The total volume is 350 ml. The void volume of the device is 139 mL.

Study design:

- 5 treatment sessions at weekly intervals (week 1-5) with a treatment duration of 60 min
- Blood flow of 30mL/min, anticoagulation by standard heparin
- Technical performance was investigated by repeated measurements of cellular blood counts, complement factor C3a as well as different cell surface markers by flow cytometry

Gating strategy FOXP3 staining (intracellular staining kit: eBioscience, San Diego, USA):



CONCLUSIONS

- The novel semi-selective device Immunopure® has been shown to be highly biocompatible and technically effective.
- The material is characterized by high removal capacities for platelets, granulocytes and monocytes. Lymphocytes and red blood cells remained substantially constant.
- Flow cytometry data revealed significant reductions of $CD10^+$ granulocytes, $CD14^+$ monocytes, $CD62L^+$ cells, $CD11b^+$ cells, $CD3^+HLADR^+$ cells, $CD3^+TCR\gamma\delta^+$ cells while there was only little impact on $CD3^+CD4^+$ and $CD3^+CD8^+$ cells.
- $CD4^+CD25^+FoxP3^+$ cells decreased at the end of the treatment.
- There were no long-term impacts on the different cell populations.

ACKNOWLEDGEMENTS

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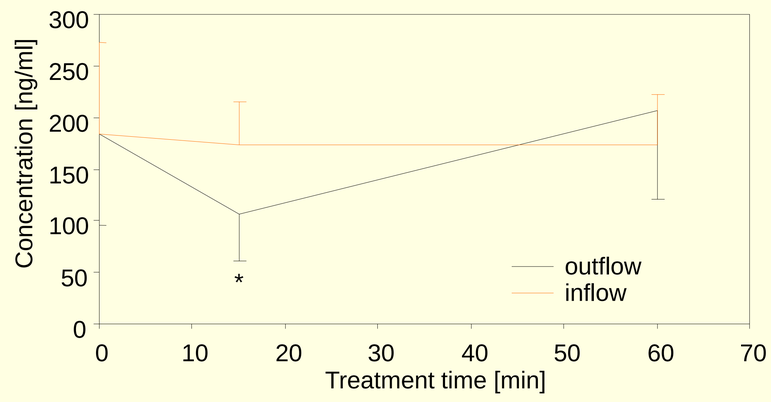
RESULTS

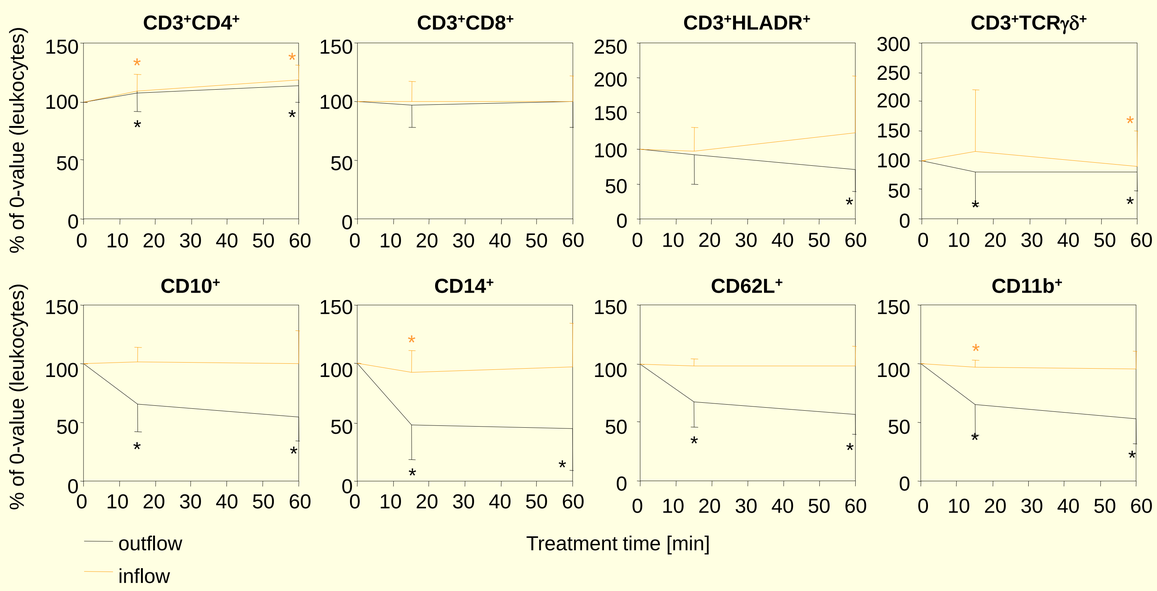
Time course during the treatment: Complement factor C3a



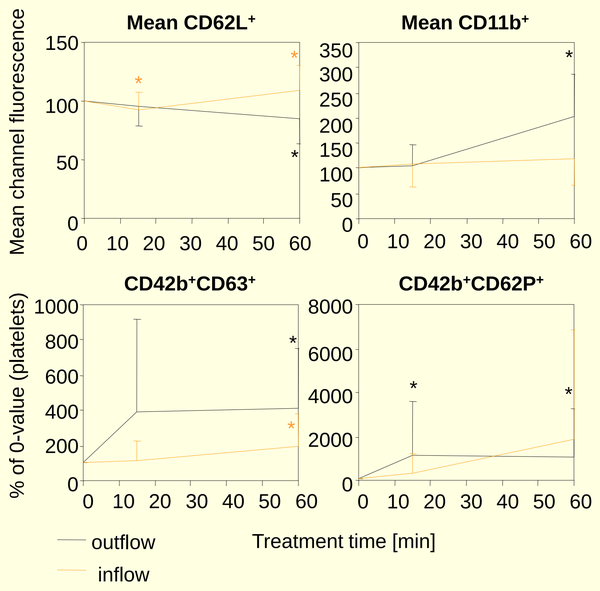
C3a values significantly decreased after 15 min of treatment in the outflow line of the device in comparison to the 0 min-values, possibly due to an adsorption of complement fragments to the adsorber material. After 60 min, levels of complement factor C3a were not significantly increased suggesting a high biocompatibility of the column material.

Parameter	0 min	15 min inflow	15 min outflow	30 min inflow	30 min outflow	60 min inflow	60 min outflow	end 65 min
Hematocrit	0.39 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.36 ± 0.03	0.37 ± 0.03
Erythrocytes	100.00 ± 0.00	100.39 ± 0.70	100.68 ± 0.51	100.08 ± 0.59	100.57 ± 0.40	100.33 ± 0.57	100.40 ± 0.72	100.46 ± 0.59
Leukocytes	100.00 ± 0.00	97.65 ± 3.31	69.81 ± 18.94	94.81 ± 5.13	59.03 ± 18.25	97.45 ± 9.15	59.54 ± 13.21	99.64 ± 8.36
Monocytes	100.00 ± 0.00	90.06 ± 15.43	51.12 ± 26.50	87.30 ± 16.81	37.29 ± 20.35	91.29 ± 12.69	34.15 ± 15.74	94.53 ± 21.07
Neutrophils	100.00 ± 0.00	97.86 ± 4.73	66.52 ± 22.09	93.82 ± 6.27	53.16 ± 22.95	96.40 ± 11.25	52.31 ± 19.10	99.01 ± 10.90
Eosinophils	100.00 ± 0.00	88.35 ± 17.70	51.52 ± 24.03	109.55 ± 25.74	49.76 ± 29.40	108.37 ± 31.02	69.91 ± 36.89	107.13 ± 24.21
Basophils	100.00 ± 0.00	97.80 ± 37.69	65.20 ± 26.73	114.62 ± 42.68	106.29 ± 76.02	115.38 ± 38.37	123.51 ± 75.09	136.38 ± 37.56
Lymphocytes	100.00 ± 0.00	99.79 ± 6.84	96.06 ± 7.45	99.44 ± 10.52	93.83 ± 9.55	105.87 ± 17.07	99.38 ± 16.12	103.94 ± 13.91
Platelets	100.00 ± 0.00	98.88 ± 2.80	20.33 ± 15.32	94.61 ± 3.15	47.69 ± 33.76	94.03 ± 4.88	73.93 ± 35.30	92.14 ± 6.11

Cellular blood counts showed that platelets, monocytes and neutrophil granulocytes were effectively reduced during the treatment at the column outlet. In contrast, lymphocytes were only moderately depleted, while red blood cells were not influenced by the device.



Flow cytometry data revealed significant reductions of $CD10^+$ granulocytes, $CD14^+$ monocytes, $CD62L^+$ cells, $CD11b^+$ cells, $CD3^+HLADR^+$ cells, $CD3^+TCR\gamma\delta^+$ cells while there was only little impact on $CD3^+CD4^+$ and $CD3^+CD8^+$ cells.



The high biocompatibility of the column material was also reflected by a relative slight decrease of CD62L and a relative low increase of CD11b mean channel fluorescence.

$CD63^+$ and $CD62P^+$ activated platelets were significantly increased after the treatment.

Parameter	0 min	15 min inflow	15 min outflow	60 min inflow	60 min outflow
$CD4^+CD25^+$	100.0 ± 0.0	**105.7 ± 17.0**	**121.1 ± 29.7**	**117.6 ± 28.2**	**118.2 ± 44.9**
$CD4^+CD25^{+interm.}$	100.0 ± 0.0	**106.5 ± 18.0**	**122.3 ± 31.5**	**118.4 ± 30.0**	**124.0 ± 44.0**
$CD4^+CD25^{+high}$	100.0 ± 0.0	104.4 ± 29.0	100.9 ± 29.9	97.6 ± 34.1	**85.5 ± 34.9**
$CD4^+CD25^+FoxP3^+$	100.0 ± 0.0	104.4 ± 27.0	114.6 ± 36.7	92.8 ± 35.8	**83.1 ± 45.5**
$CD4^+CD25^{+interm.}FoxP3^+$	100.0 ± 0.0	102.9 ± 33.4	111.7 ± 44.3	93.6 ± 53.1	**80.7 ± 59.4**
$CD4^+CD25^{+high}FoxP3^+$	100.0 ± 0.0	**104.3 ± 26.3**	115.8 ± 36.5	93.7 ± 39.0	**76.5 ± 38.4**

$CD4^+CD25^+$ and $CD4^+CD25^{+interm.}$ cells significantly increased during the treatment. In contrast, $CD4^+CD25^{+high}$ and $FoxP3^+$ cells decreased at the end of the treatment. These results may reflect a slight T cell activation due to the column material. However, the short-term influence of the treatment on T_{regs} seems to be only marginal.