Influence of NaCl 0.9% Infusion during Plasmapheresis on IgG Content in Plasma

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Keywords

Apheresis · Immunoglobulin G · NaCl 0.9% infusion · Plasmapheresis

Summary

Background: The aim of this study was to evaluate the influence of an infusion of NaCl 0.9% 500 ml during preparatory plasmapheresis or apheresis on the immunoglobulin G (IgG) content in separated plasma. **Methods:** 32 donors of plasma were studied in a crossover design after informed consent on one day without NaCl 0.9% 500 ml during apheresis and on another day with infusion of NaCl 0.9% 500 ml during apheresis. Infusion of NaCl 0.9% 500 ml was given step by step in divided doses after each cycle through the harness set of the Haemonetics® plasma collecting system 2 (PCS2). Concentrations of IgG in serum and in plasma were measured by an immunoturbidimetric assay. Percentages of IgG concentrations in plasma were calculated by dividing the IgG concentration in plasma by the mean serum IgG concentrations ($\times 100$). **Results:** Without infusion of NaCl 0.9% 500 ml, the mean percentage of IgG in separated plasma was $85.5 \pm 2.3\%$ while it was $80.5 \pm 3.4\%$ when NaCl 0.9% 500 ml was given. The difference between the two samples was statistically highly significant ($p < 0.001$). **Conclusion:** We conclude that the gradual infusion of NaCl 0.9% 500 ml during apheresis causes a statistically highly significant difference of IgG content in separated plasma.

Schlüsselwörter

Apherese · Immunoglobulin G · NaCl-0,9%-Infusion · Plasmapherese

Zusammenfassung

Hintergrund: Das Ziel dieser Untersuchung war, den Einfluss einer Infusion von NaCl 0,9% 500 ml während der präparativen Plasmapherese oder Apherese auf den Immunoglobulin-G(IgG)-Gehalt im getrennten Plasma zu bestimmen. **Methoden:** Das Plasma von 32 Spendern wurde in einem Crossover-Design nach schriftlicher Einverständniserklärung an einem Tag ohne NaCl-Infusion während der Apherese und an einem anderen Tag mit einer Infusion von 500 ml NaCl 0,9% während der Apherese untersucht. Die Infusion von NaCl 0,9% 500 ml wurde allmählich in geteilten Dosen nach jedem Zyklus über das Einmalschlauchsystem (Harness Set) des Haemonetics® Plasma Collecting System 2 (PCS2) verabreicht. Die IgG-Konzentrationen im Serum und im Plasma wurden mit einem immunturbidimetrischen Test gemessen. Der prozentuale Anteil der IgG-Konzentrationen im hergestellten Plasma wurde durch Division durch die mittleren Werte (Mittelwerte) der IgG-Serumkonzentrationen berechnet ($\times 100$). **Ergebnisse:** Ohne Infusion von NaCl 0,9% 500 ml betrug der prozentuale Anteil von IgG im Plasma $85,5 \pm 2,3\%$ der mittleren IgG-Serumkonzentrationen; mit NaCl 0,9% 500 ml war der Anteil $80,5 \pm 3,4\%$. Der Unterschied zwischen den beiden Stichproben war statistisch hoch signifikant ($p < 0,001$). **Schlussfolgerung:** Wir schlussfolgern, dass die allmähliche Infusion von NaCl 0,9% 500 ml während einer Apherese zu einer statistisch hoch signifikanten Verminderung des IgG-Gehalts im Produktplasma führt.

Introduction

Infusion of NaCl 0.9% 500 ml is an accepted practice to prevent hypovolemic events during apheresis with the Haemonetics® Plasma Collecting System 2 (PCS2; Haemonetics GmbH, München, Germany)).

The advantages of this volume treatment are apparently less hypotensive reactions during preparatory plasmapheresis and less symptoms of tiredness after plasma donations. The disadvantage may be a dilution effect of infused saline solution on the separated plasma. However, there are no published data available thus far if there is a significant effect of infusion of NaCl 0.9% 500 ml on the quantity of IgG concentrations in separated plasma. The present study therefore aimed at evaluating the influence of an infusion of NaCl 0.9% 500 ml, gradually given through the harness set of the PCS2 during apheresis, on the IgG concentration of separated plasma.

The primary endpoint was to determine the relative content of IgG in percent in separated plasma in relation to the mean serum concentration of IgG during apheresis sessions on a day without infusion of NaCl and on a day with infusion of NaCl 0.9% 500 ml, but not to determine the absolute changes of IgG in plasma because these changes are dependent on changes of IgG concentrations in serum of donors after repeated plasma donations. In contrast, the percentage IgG in separated plasma in relation to the mean serum concentrations of IgG during a plasmapheresis session of less than 1 h, is not dependent on changes of IgG concentrations in serum.

Participants and Methods

Participants

32 donors (16 men, 16 women) of plasma were enrolled in the study. Details of the participants are given in the table 1.

All subjects were in good health on the basis of medical history, physical examination and hematological screening, and had no laboratory evidence of hepatitis B, hepatitis C, HIV infection, or syphilis.

Volunteers were excluded if they had any contraindication to donate plasma according to the regulations of the German Medical Association [1] or to the Guide to the Preparation and Quality Assurance of Blood Components of the Council of Europe [2].

Design

The study was performed according to national law in Germany and met the standards of the Declaration of Helsinki. Written informed consent was obtained from each participant.

The study was done prospectively, in a single-center, crossover design.

A crossover test design was chosen to determine the effect of a NaCl 0.9% 500 ml infusion on the one hand and to exclude systematically definite side effects on the other hand. These are: a carry-over or residual effect, which implies a too short time period between the treatments with and without NaCl infusion, and a time effect which indicates a generally difference between the first and the second treatment. All 32 participants were studied on two aphereses on two separate days. Between the two aphereses at least 48 h had to pass. 16 subjects were first treated without NaCl infusion during apheresis on the first day. On the second apheresis

Table 1. Clinical data (N = 32, 16f/16m)

	Age, years	Weight, kg	Hematocrit, %
Mean	31	70	40
SD	14	12	3
Max	62	93	46
Min	18	50	35

procedure, an infusion of NaCl 0.9% 500 ml (free flex®; Fresenius SE, Bad Homburg, Germany) was given step by step during apheresis through the harness set of the plasma collecting system after each cycle. The other 16 participants first received an infusion of NaCl 0.9% 500 ml during apheresis and the second apheresis procedure was done without NaCl infusion.

Participants should have a light meal within 4 h and 1.5 l fluid intake before apheresis on the days of study. They additionally received 200 ml mineral water and were interviewed and examined within 30 min before apheresis.

Aphereses were performed with the Haemonetics PCS2. Sodium citrate 4% anticoagulant solution (Haemonetics) was added to venous blood during the draw phase in a ratio of 1 part sodium citrate 4% solution to 16 parts of whole blood. Volume of separated plasma was 760 ml. Running parameters of the aphereses, such as volume of separated plasma, processed blood volume (PBV), duration of the procedure, number of cycles, consumption of NaCl 0.9% and of sodium citrate 4% anticoagulant solution, were recorded in a protocol.

After venipuncture, samples of 5 ml blood were taken from the tube of the fistula needle first for testing for IgG concentrations in serum before apheresis.

At the end of aphereses 10 ml of blood was withdrawn from the tube of the fistula needle to clear the tube. After that a second sample of 5 ml blood was taken for IgG concentration in serum after aphereses. Additionally, samples of 5 ml plasma were taken from the separated plasma for IgG concentrations in plasma.

Blood samples were centrifuged after 30 min at +20 °C at a speed of 4,000 rpm for 15 min (Hettich Centrifuge Rotina 38, g-force 2737) and serum was taken for the determination of IgG concentrations in the serum.

Serum samples were stored at 4–7 °C and plasma samples at <–30 °C until analysis.

Immunoglobulin G Analysis

IgG levels in serum and in plasma were measured by an immunoturbidimetric assay with an Olympus AU640 (Olympus, Hamburg, Germany). The assays were performed in the Institute of Clinical Chemistry of the German Red Cross East at Chemnitz. The test was validated for serum and plasma [3, 4].

Statistical Analysis

Details of the participants and results of IgG concentrations in serum before and after aphereses, IgG concentrations in plasma, and data of aphereses were listed in a MS Excel table (Microsoft, Redmond, WA, USA). Mean values, standard deviations and maximal and minimal values were calculated.

The percentages of IgG concentrations in separated plasma were calculated by dividing the IgG concentration in plasma by the mean serum IgG concentrations according to the following formula:

$$\% \text{IgG} = \frac{\text{IgG concentration in plasma} \times 100}{\text{mean IgG serum concentration}} \quad (1).$$

$$\begin{aligned} \text{Mean IgG serum concentration} \\ = (\text{first IgG in serum} + \text{second IgG in serum}) / 2 \end{aligned} \quad (2).$$

Table 2. Results

	Without NaCl	With NaCl	p
IgG in plasma, %	85.5 ± 2.3	80.5 ± 3.4	<0.001
IgG in Plasma / IgG in serum, %	80.3 ± 2.5	73.9 ± 2.9	
Time, min	46 ± 6	53 ± 6	
Clearance IgG, ml/min	14 ± 2	12 ± 2	
Cycles	4.7 ± 1	4.4 ± 1	
Processed blood volume, ml	2,077 ± 218	2,070 ± 185	
Citrate, ml	130 ± 12	130 ± 14	

The percentage (Q) of the IgG concentration in separated plasma in relation to the first IgG concentration in serum of the blood of the donor before aphereses was calculated in excel according to the formula:

$$Q\% = \frac{\text{IgG concentration in plasma} \times 100}{\text{first IgG concentration in serum}} \quad (3).$$

The clearance or elimination of IgG by apheresis was calculated according to this formula:

$$\text{Clearance}_{\text{IgG}} = \frac{\text{IgG concentration in plasma} \times 760 \text{ (ml)}}{\text{mean IgG concentration in serum} \times \text{time (min)}} \quad (4).$$

Thus the elimination of IgG by apheresis was determined according to the clearance formula (excreted amount divided by the area-under-curve (AUC), AUC calculated by the trapezoid rule). This formula is not only used to calculate the renal clearance in nephrology but also to determine the clearance of extracorporeal artificial organs or the elimination of drugs by various dialysis procedures [e.g. 5].

With respect to the specific study design, statistical analysis was performed by a Wilcoxon signed-rank test for paired dependent samples [6, 7].

Results

All 32 included donors completed the study. The results are summarized in table 2.

Without giving an infusion of NaCl 0.9% during aphereses, the average percentage of IgG in separated plasma in relation to the mean IgG serum concentrations was 85.5 ± 2.3%, and it was 80.5 ± 3.4% when NaCl 0.9% was given. The difference of 5% was statistically highly significant (p < 0.001). A significant carry-over effect as well as a time effect were not detected.

In addition we found an noticeable difference in the percentage of IgG in separated plasma in relation to IgG serum concentration before apheresis (without and with treatment). The mean time for aphereses with infusion of NaCl 0.9% 500 ml was longer by 7 min than that for standard aphereses without NaCl. When NaCl 0.9% 500 ml was given, the plasma clearance of IgG by apheresis was reduced by about 2 ml/min.

We observed no important differences in the number of cycles, the volumes of processed blood and the consumption of sodium citrate 4% anticoagulant solution.

Two participants had a lightly hypotensive event after apheresis without infusion of NaCl 0.9% 500 ml. These events were reversible within 45 min. There were no serious or persisting adverse reactions.

Discussion

To our knowledge, this is the first report on the influence of the infusion of NaCl 0.9% 500 ml during automated apheresis using a PCS2 on the IgG concentration in separated plasma.

We found a statistically highly significant difference of 5% in the percentage of IgG concentrations in separated plasma in relation to the mean IgG serum concentrations between standard aphereses without and those with infusion of NaCl 0.9% 500 ml. This main result was supplemented by noticeable differences in the percentages of IgG in separated plasma in relation to the IgG in serum before apheresis (without and with NaCl 0.9% 500 ml) and a reduction of the clearance of IgG by apheresis when an infusion of NaCl 0.9% 500 ml was applied (table 2). Our results show that the infusion of NaCl 0.9% 500 ml, given step by step through the harness set of the PCS2 during preparatory plasmapheresis, is accompanied by a dilution effect in the separated plasma. The by 7 min longer duration of apheresis with NaCl infusion can be explained by the time which is necessary for the gradual infusion of NaCl 0.9% 500 ml after each cycle. Neither the number of cycles, nor the volumes of processed blood nor the consumption of sodium citrate 4% anticoagulant solution were significantly affected by infusion of NaCl 0.9% 500 ml.

Our results suggest that there may be a similar effect of infusion of NaCl 0.9% 500 ml during preparatory plasmapheresis on other serum proteins such as factor VIII (anti-hemophilic factor). Factor VIII levels in plasma of healthy donors were more variable than IgG concentrations [8]. During apheresis factor VIII levels in plasma were dependent on concentrations of sodium citrate concentrations [9, 10]. We did not find any study on the influence of infusion of NaCl 0.9% 500 ml during apheresis on factor VIII yield of the resulting plasma. However, the question remains if there is any effect of infusion of NaCl 0.9% 500 ml on factor VIII levels.

In agreement with one earlier study [10], we assume that the 14.5% decrease of the mean IgG concentrations in serum minus the IgG concentrations in separated plasma in standard aphereses without infusion of NaCl 0.9% 500 ml is caused by the addition of citrate 4% anticoagulant solution. We calculated that 14.5% of 760 ml = 110 ml of citrate 4% solution remained in the separated plasma, and a minor rest of 130 ml – 110 ml = 20 ml citrate 4% solution was infused with

returned blood cells into the body of the donor. Further studies with direct measurement of citrate in separated plasma, serum and urine are necessary to determine the disposition of citrate anticoagulant solution in the body of the donor and to confirm effects on mineral metabolism and bone [11].

We concluded that there was a statistically highly significant difference of 5% and a dilution effect in the IgG content in separated plasma when NaCl 0.9% 500 ml was given during preparatory plasmaphereses. However, no general guideline could be derived from our results for or against the infusion of NaCl 0.9% 500 ml during aphereses. We did not change our prescription to the donor to take a light meal within 4 h before aphereses and to have 1.5–2 l fluid (before aphereses). In order to prevent hypovolemic events during preparatory plas-

maphereses we give NaCl 0.9% 500 ml in divided doses after each cycle through the harness set of the PCS2 to all first plasma donors. At repeated aphereses an infusion of NaCl 0.9% 500 ml is given when clinically indicated.

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Disclosure

The authors declared no conflict of interest.

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Erratum

In the article

Evers J, Betz J, Blankenburg T, Engelen T, Galle G, Hansen M, Laitinen T, Taborski U: Influence of NaCl 0.9% Infusion during Plasmapheresis on IgG Content in Plasma. Transfus Med Hemother 2010;37(4):191–192.

a wrong storage temperature for plasma samples was given on page 192, right column.
The respective paragraph should be read as follows:

‘Serum samples were stored at 4–7 °C and plasma samples at <–30 °C until analysis.’

We apologize for this mistake.