A model comparing how rapidly transfusion of solvent detergent plasma restores clotting factors versus infusion of albumin-saline

Petra Jilma-Stohlawetz, Friedrich W. Kursten, Michaela Horvath, Gerda Leitner, Jana List, Jana Marcek, Peter Quehenberger, Michael Schwameis, Johann Bartko, Bernd Jilma

* Corresponding author. Department of Clinical Pharmacology, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria. Tel.: +43 (0)1 404002981; fax: +43 (0)1 404002998. E-mail address: Bernd.Jilma@meduniwien.ac.at (B. Jilma).

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1. Introduction

A recent randomized, double blind trial (hereafter referred to as the core study) demonstrated the bioequivalence between a pharmaceutical grade, universally applicable, pathogen safeguarded plasma (Uniplas LG) and its parent compound (Octaplas LG) in healthy individuals. This core trial also demonstrated the absence of haemolytic transfusion reactions after transfusion of universal plasma [1]. However, the changes in coagulation factor levels and inhibitors throughout the procedure of plasmapheresis (PPh; 600 mL of plasma was removed) and subsequent transfusion of 1200 mL plasma was rather small. For example, the
observed decrease in fibrinogen averaged 13% after PPh, although a 25% decrease in plasma coagulation factors was expected. Likewise, fibrinogen increased by only 6% after transfusion of 1200 mL plasma (Fig. 1), although a greater rise in fibrinogen was expected based on the concentration of clotting factors in the plasma products (90% of normal). As both plasma products were active treatments and similar in terms of protein content and plasma colloid-osmotic pressure, the active-control design of the study did not provide any control to explore possible changes in plasma proteins throughout such a procedure.

We hypothesized that fluid redistribution may have occurred following PPh and/or plasma transfusion, and was responsible for the lower than expected observed effects in both directions. To address this issue experimentally, the core study was augmented by a third control period (extension arm), in which volunteers received an infusion of 1200 mL albumin solution after PPh, instead of plasma. Hence, the aims of the extension study and the overall analysis of the three periods were to validate the experimental model and setup to increase assay sensitivity, in order to investigate the true capacity of these plasma products to restore coagulation factor levels and inhibitors after experimental plasma depletion.

As albumin supports plasma colloid-osmotic pressure, it is often administered as a resuscitation fluid [2]. However, there is no clear consensus over the choice of resuscitation fluid. Fluid resuscitation with albumin was associated with higher mortality rates than was resuscitation with saline in a post-hoc study of patients with traumatic brain injury [3]. However, the use of albumin-containing solutions was associated with lower mortality compared with other fluid resuscitation regimens in a meta-analysis of studies with septic patients [4]. Finally, no substantial difference between crystalloids and colloids was seen in any important patient-centred outcome in 6997 critically ill patients [5]. Thus, it was also of interest to compare the effects of an infusion of 1200 mL albumin solution with plasma on coagulation parameters from the perspective of albumin as a potential resuscitation fluid.

2. Subjects and methods

The core study and subsequent amendments were approved by the Ethics Committee of the Medical University of Vienna and the national authority (Österreichische Agentur für Gesundheit und Ernährungssicherheit: AGES), and was conducted in compliance with the regulations of Good Clinical Practice (CPMP/ICH/135/95), and the Declaration of Helsinki 2008. All subjects re-consented in writing before inclusion into the extension study arm.

2.1. Study design and subject population

The objectives of this extension study were to compare the recovery of clotting factors and inhibitors of Uniplas LG and Octaplas LG, reported in the core study, with that of albumin solution (primary objective) and to compare the safety and tolerability of the pathogen safeguarded plasmas with those of an albumin infusion (secondary objective).

Subjects with blood group O were excluded, because they were not expected to be at risk of incompatibility after transfusion of Uniplas LG. Exclusion criteria and enrolment criteria for the extension arm (third period) were the same as for the core study [1], with the additional requirement that all subjects entering the extension arm had to have completed the core study. Thus, only subjects who had received full amounts of both plasma products (i.e., 1200 mL Uniplas LG and 1200 mL Octaplas LG [both Octapharma PPGmbH, Vienna, Austria]) in the core study were eligible for the extension study arm. This subgroup of eligible, healthy subjects underwent similar procedures as in the previous core study where they had received the two plasma products in a randomized sequence (randomization faxed from a central...
contract research organization office) after plasma removal of 600 mL in a cross-over design. The characteristics of the two products have been described previously [6–10]. According to the Austrian guidelines a maximum of 700 mL (without anticoagulation) can be removed by plasmapheresis dependent on the donor’s weight; an equivalent removal of volume before replacement would result in cardiovascular adverse events, like hypotension and circulatory collapse. While the trial was double blind for the first two periods, the third period was open label.

2.2. Study treatments and visits

After a standard PPh of 600 mL plasma, subjects received 750 mL albumin 5% + 450 mL 0.9% NaCl (total volume of 1200 mL and a final albumin concentration of 3.125% to mimic albumin contents of the transfused plasma products) according to the same procedures as in the core study; these two solutions were mixed prior to infusion. An identical infusion speed was used (i.e., < 1 mL/kg BW/min). Subjects reported to the clinic again for a short examination and blood sampling 24 hours and 7 days after the infusion of albumin solution.

All tests were performed according to standardized, routinely used laboratory assays, in ISO certified laboratories of the hospital.

2.3. Biochemical endpoints

Recovery was assessed by coagulation and haemostatic parameters (activated partial thromboplastin time [APTT; expressed in s], prothrombin time [PT; expressed in %], fibrinogen, FII, FV, FVII, FVIII, FIX, FX, FXI, protein S and plasmin inhibitor), as previously described [1]. The parameters were evaluated before PPh, after the end of PPh, as well as 15 minutes, 2 hours and 24 hours after infusion to investigate short (15 minutes after end of transfusion) and longer recovery times (2 hours, 24 hours). Analysis of recovery was done for exploratory purposes only and no primary efficacy variable was defined.

2.4. Safety endpoints

The following safety endpoints were determined: (1) parameters of haemolysis: haemoglobin (Hb), haptoglobin, free Hb and indirect bilirubin on a Hitachi Analyzer using Roche Reagents (Sysmex G.m.b.H, Vienna, Austria); (2) immune haematology: direct antiglobulin test (DAT) performed on all blood samples with the DiaMed-ID Micro Typing System (DiaMed, Cresier, Switzerland) (not measured 15 minutes post-transfusion in periods 1 and 2), and Complement activation: C3c, CH50 and C4 (Dade Siemens Diagnostics, Vienna, Austria); (3) haematological parameters: red blood cells (RBC), haematocrit, white blood cells (WBC) and platelets were quantified on a Sysmex X2100 cell counter (Roche, Vienna, Austria) (RBC, WBC and platelets at baseline, before-PPh and at Visit 8 only). Clinical chemistry and urine analysis were also carried out.

2.5. Statistical methodology

No formal separate sample size estimation was carried out for the extension study, but based on the observed variance and the estimated effect size of approximately 25% in the albumin arm, it was estimated that a number of > 12 subjects should suffice for our study aims. In addition to raw values of coagulation and haemostatic parameters, absolute and relative differences compared to baseline values (measured before any intervention, i.e., before the start of the preceding PPh at visit 2, visit 5 and visit 10, respectively) were calculated for each of the three treatment arms. Moreover, treatment group ratios of relative changes were also calculated. Descriptive statistics for each parameter at post-transfusion time are provided and a time course of differences is presented, including 95% confidence intervals (CIs). Statistical characteristics of each parameter are presented by treatment periods.

The change from the values measured before the start and after the end of PPh to maximum (or minimum where appropriate) was calculated for all treatment periods and the Wilcoxon signed rank test was applied for differences D1 – D2 and D2 – D3, where D1 is the value of a parameter in the Uniplas LG treatment period, D2 is the value of a parameter in the Octaplas LG treatment period, and D3 is the value of a parameter in the albumin treatment period. In addition, the Wilcoxon signed rank test was also applied for differences D11 – D12 to optimize interpretation of study results.

Although all evaluations and analyses performed using the third treatment period were done in an exploratory manner, p-values for the ratio of relative differences (i.e., Uniplas LG/albumin solution, Octaplas LG/albumin solution, Uniplas LG/Octaplas LG) were calculated. As the relative difference of 15 minutes after transfusion to post-PPh reflects quick recovery of coagulation factors, a focus was on the p-value of this particular relative difference.

The comparison of the Hb change between treatment arms was analyzed by the analysis of variance model with treatment effect. Using the square root of residual mean squares as an estimate of the variance, two-sided 95% CIs for treatment differences of Hb change were calculated. Any p-values were calculated only for exploratory purposes. Other safety endpoints were analyzed descriptively between the treatments, together with mean treatment differences estimated along with 95% CIs for continuous parameters. The statistical analysis was carried using SAS software, version 9.1.

3. Results

Between 30th of January and 12th of June 2009, 30 subjects (17 males, 13 females) were randomized and received at least one study treatment in the core study. Twenty-five subjects completed both treatment periods, of whom 14 (9 males, 5 females) agreed to participate in the extension study arm and were re-screened between 21st of September and 14th of October 2009. Their mean age (± standard deviation [SD]) was 35 ± 8 years (range: 23–48 years) and mean height, weight and body mass index were 179 ± 9 cm, 76 ± 15 kg and 23.9 ± 3.9 kg/m², respectively.
As all 14 subjects had received the full doses (1200 mL – equivalent to 16.3 ± 3.1 mL/kg) of plasma products in the core study, they received the same dose of albumin solution (16.3 ± 3.1 mL/kg).

All 14 subjects completed the core study and its extension; no relevant protocol deviations were reported in any subject in any of the three treatment periods. Thus, no subject was excluded from the efficacy analysis and the safety and per protocol populations were identical.

3.1. Efficacy

3.1.1. Coagulation factors and global coagulation tests

A dilution effect occurred, due to the volume of fluid infused. This is best seen in the albumin solution treatment period, in which a solution with similar plasma colloid-osmotic pressure to that of plasma, but devoid of coagulation factors and inhibitors, was infused. This dilution effect lasted for about 24 hours or even longer and is best exemplified by the plasma concentrations of those coagulation factors with long half-lives, such as fibrinogen or FII (prothrombin; Figs. 1 and 2).

Figs. 1–4 demonstrate a very rapid recovery (within 15 minutes) of coagulation factors after plasma transfusion, so that relative differences of clotting factors to baseline were close to 0 or positive at all measured time points. In contrast, they were consistently lower than baseline after infusion of albumin solution (Figs. 1–4). Both plasma products almost completely restored baseline fibrinogen levels, whereas the infusion of albumin solution did not (p = 0.0001 plasma products vs. albumin).

Plasma levels of FII (Fig. 2), a coagulation factor of the common terminal pathway, with a half-life of 48–60 hours, showed a pattern similar to fibrinogen. After an initial decrease due to the removal by plasmapheresis, prothrombin levels continuously increased and were close to baseline 15 minutes after plasma transfusion, whereas they were consistently below baseline after infusion of albumin solution (p = 0.0001 plasma products vs. albumin). Changes in FV and FX levels resembled those of FII (data not shown).

Factor VII, a coagulation factor of the extrinsic pathway, also decreased initially after PPh and increased again along with the transfused plasma (Fig. 3). The early effects of infusion of albumin solution up to 2 hours were comparable to the response seen with the other coagulation factors. However, 24 hours after infusion of albumin solution, FVII levels were comparable to those in the plasma transfusion periods, due to the relatively short regeneration time of FVII (<7 hours).

Plasma transfusion also quickly restored FXI, a coagulation factor of the intrinsic pathway, after plasmapheresis, whereas albumin solution induced a dilution effect that lasted 24 hours or more (Fig. 4). Again, the dilution effect of FXI was longer than for FVIII and FIX, because of their shorter half-lives (data not shown).

After transfusion of the plasma products, the variation in PT reflected the characteristic pattern observed in coagulation factors of the extrinsic pathway (Fig. 5). A discrete initial decrease (less than 10%) was seen following PPh due to the removal of coagulation factors determining PT (i.e.: FII, FV, FVII, FX ), which was followed by a small increase 15 minutes after plasma transfusion (levels were close to preapheresis levels). Prothrombin time stabilized and remained almost constant thereafter. In the albumin treatment period, the initial decrease in PT due to plasmapheresis was further accentuated. Prothrombin time was comparable to the plasma transfusion periods after 24 hours, mainly because the applied PT assay is very sensitive to FVII (Fig. 2).

aPTT is another global coagulation test which measures the intrinsic coagulation pathway (it reflects the activity of FVIII, FIX, FXI and FXII). The loss of coagulation factors did not translate into a remarkable change in aPTT during any of the three treatment periods (data not shown). The transfusion of plasma products decreased the aPPT by
2–3 s, while infusion of albumin solution increased the aPPT by 1 s at 15 minutes.

3.1.2. Protein S and plasmin inhibitor

Protein S, a cofactor for protein C, and plasmin inhibitor, a fibrinolysis inhibitor, were also measured in the three treatment periods (visit 2, visit 5 and visit 10).

Although mean values of protein S were consistently within normal range in the plasma treatment periods, recovery of protein S after PPh was somewhat slower than that seen in coagulation factors. As expected, in the albumin treatment period, protein S continued to decrease until after the infusion of albumin solution ($p = 0.0001$ plasma products vs. albumin).

The same observation was made for plasmin inhibitor with, however, lower relative differences to pre-PPh and post-PPh in the plasma treatment periods and a more discrete dilution effect caused by albumin (data not shown).

3.2. Safety results

3.2.1. Markers of a haemolysis transfusion reaction (HTR)

As expected, neither the plasma products nor albumin solution induced a HTR, and not even a single positive DAT test was observed in any of the three treatment periods. No biological signs of HTR were detected in any of the three treatment periods. All means of the tested markers, namely Hb, haptoglobin, free Hb, indirect bilirubin, complement
activation and DAT were consistently within the normal range. Variations were similar between the three treatment periods.

3.2.2. Adverse events

In total, 35 adverse events were observed in 11 subjects during the three treatment periods, of which 4 occurred during the albumin solution treatment period (1 gastrointestinal disorder, 1 musculoskeletal and connective tissue disorder, and 2 nervous system disorders). None of the adverse events in the albumin solution treatment period was considered to be possibly or probably related to treatment.

Abnormal laboratory values occurred with the same frequency during the three treatment periods; they were all clinically irrelevant.

4. Discussion

Uniplas LG is a blood group-independent, i.e., universally applicable, pathogen safeguarded, pooled plasma and can therefore be administered irrespective of the recipient’s blood group. In earlier clinical studies conducted in patients with pathological conditions, universal plasma was equivalent to routinely used solvent/detergent (S/D) treated plasma in terms of safety, tolerability and efficacy [10,11].

As part of the product’s clinical development, a study has also been conducted in healthy subjects, which compared the safety and tolerability of Uniplas LG with an AB0 compatible parent plasma (Octaplas LG) in healthy volunteers [1]. The ‘LG’ products denote further developments in manufacturing of their respective earlier versions. The ‘LG’ process was introduced to remove prions from S/D plasma, by means of a specifically designed, affinity ligand gel (LG) column, and thereby reduce the risk of developing variant Creutzfeldt-Jacob disease from transfused plasma. A recent study done in the UK has demonstrated that the affinity chromatography procedure used in Octaplas LG manufacture did not adversely affect the known haemostatic quality of the parent product [12].

We have previously demonstrated that both plasma products were equivalent with respect to safety, tolerability and efficacy and ruled out the occurrence of HTRs after transfusion of the universal version [1]. Although their bioequivalence was established, our analysis indicated smaller than expected changes in coagulation factors. The active-control design of the study did not provide any control to explore a possible fluid redistribution and/or dilution effect following PPh and plasma transfusion.

To address this issue, the study was extended by a third period, during which subjects received an infusion of albumin solution as a control arm. Because of its protein content, an albumin solution accounts for approximately 75% of plasma colloid-osmotic pressure. It was therefore judged more appropriate than physiological saline solution, which lacks plasma colloid-osmotic pressure properties.

Infusion of albumin solution (1200 mL) further decreased clotting factors such as fibrinogen, FII, FVII and FXI (Figs. 1, 3, 4 and 5) after PPh, usually until 15 minutes post-infusion. The dilution effect lasted ≥24 hours for clotting factors with a long half-life such as fibrinogen, the leading determinant in dilution coagulopathy [13].

Plasmapheresis decreased the levels of coagulation factors and inhibitors to a similar extent in all three periods. However, plasma transfusion of either product rapidly increased the plasma levels of coagulation factors and inhibitors despite the dilution effect. Mean values of coagulation factors were consistently significantly higher in the plasma product treatment periods than during the albumin treatment period (Figs. 1–4). Baseline values of coagulation factors were generally reached within 15 minutes to 2 hours following the end of plasma transfusion, indicating a quick recovery of coagulation factors.

These changes were followed closely by the changes in both global coagulation tests. Thus, the changes in PT (Fig. 5)
reflected FII, FV, FVII, FX and fibrinogen activities. However, aPPT showed an inverse pattern (mainly a decrease), because a decrease in aPPT indicates an increase in the activity of coagulation factors.

Our results are in line with a dilution effect of albumin on FXI and FXIII in children undergoing craniofacial surgery [14] and the correction of dilution coagulopathy by fresh-frozen plasma in patients after major surgery [15].

Although the results cannot be immediately extrapolated to clinical practice, they do have potentially interesting implications. When plasma volume is critically jeopardized, the use of a plasma transfusion as a volume substitute has been considered contraindicated [16,17]. In contrast, several conventional colloid solutions (e.g., hydroxyethyl starch [HES], dextrans, gelatins and albumin) or crystalloids are widely used for blood volume replacement therapy. Colloids are capable of rapidly restoring circulating volume with a smaller infused volume than physiological saline. Although HES solutions are increasingly preferred in the critical care setting [18] intervention in blood coagulation has limited the clinical use of some preparations [19]. Furthermore, the clinical utility of administering albumin, a colloid which is considered a near optimal resuscitation fluid because it occurs naturally in plasma [20,21], is also a matter of controversy, not least because of its cost. The discussions on the relative merits of the various colloids arise because, alongside their intravascular expansion properties, they have a varying influence on other critical parameters, notably coagulation and renal function [22].

One immediate effect of the use of crystalloid or colloid infusions in order to maintain normovolaemia in patients experiencing blood loss is the dilution of clotting factor concentrations, as indeed was seen in this trial. This has clinical consequences because, clearly, impaired coagulation is undesirable in patients with major blood loss. Even moderate dilution with colloids compromises the coagulation system in several ways, including impaired fibrinogen polymerization and decreased clot strength [23–28]. Clot firmness is recognized as a precondition for arresting bleeding [28].

Therefore, bleeding control will not be restored quickly if a patient is only treated by an infusion of a colloid, such as albumin, which is devoid of coagulation factors. Although such infusions will replace the lost volume in critical cases, without the additional use of plasma containing the appropriate coagulation factors, the necessary levels of these factors might never be achieved.

In conclusion, this experimental model is suitable for demonstrating the equivalence of two transfusion plasmas with regard to restoration of coagulation parameters versus the dilution effect of albumin solution. It helps in the interpretation of similar trials [29]. In contrast to albumin solution, which induces a fairly long-lasting decrease of coagulation factors, both plasma products promptly restored the levels of diluted coagulation factors and inhibitors.

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References


