Thawed solvent/detergent-treated plasma: too precious to be wasted after 6 hours?

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Background. Coagulopathy associated with trauma and bleeding requires early administration of haemostatic agents. Solvent/detergent-treated plasma (S/D-plasma) requires thawing and its availability for clinical use is, therefore, delayed. The long-term stability of clotting factors in thawed S/D-plasma has not been thoroughly investigated. The purpose of this study was to evaluate stability of clotting factors and inhibitors in thawed S/D-plasma stored at 4 °C for 6 days.

Materials and methods. Clotting factor levels and bacterial contamination were investigated using 20 units of S/D-plasma. Fibrinogen, factor (F) II, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII, antithrombin, von Willebrand antigen (VWF-Ag), plasmin inhibitor, protein C and free protein S were analysed over time.

Results. After 6 days of storage the results were as follows: fibrinogen 270 mg/dL (−10 mg/dL, p=0.0204), FII 75% (−5%, p<0.0001), FV 88% (−14%, p<0.0001), FVII 81% (−24%, p<0.0001), FVIII 70% (−16%, p<0.0001), FIX 96% (−8, p<0.0001), FX 92% (−1%, p<0.0001), FXI 119% (−4%, p=0.3666), FXII 94% (−2%, p=0.3602), FXIII 89% (−1%, p 0.0019), free protein S 76% (−4%, p<0.0001), protein C 96% (+1%, p=0.0371), antithrombin 92% (−3%, p<0.0001), plasmin inhibitor 29% (−4%, p<0.0299), VWF-Ag 137% (+2%, p=0.2205). FVII and FVIII showed a critical drop of more than 20% or approached the lower quality assurance threshold after storage for more than 24 hours. No S/D-plasma showed bacterial contamination.

Conclusion. All clotting factors in thawed S/D plasma remained stable for up to 24 hours when stored at 4 °C. Storage of thawed S/D plasma may improve the availability of this product in emergency situations.

Keywords: S/D-plasma, massive transfusion, plasma storage, clotting factors.

Introduction

The "lethal triad" in patients with severe haemorrhagic injuries is hypothermia, acidosis and coagulopathy[1]. Coagulopathy associated with trauma may require early haemostatic therapy. Recent results have shown that trauma-associated coagulopathy may be treated successfully if high quality plasma is available promptly in order to prevent further loss and dilution of clotting factors[2-4]. Volume resuscitation in civilian and military trauma care usually starts with administration of crystalloid or starch solutions at the site of first assessment of the patient and then packed red blood cells (PRBC) in the emergency room because these are immediately available[3] whereas plasma requires thawing which is a time-consuming process. The currently available and approved thawing devices need up to 30 minutes to thaw two or three units of plasma[4]. The availability of thawed plasma would allow an early 1:1 ratio of transfusion of red blood cells and plasma, which has been shown to be associated with reduced mortality and aggravation of coagulopathy[3,5].

Transfusion of fresh-frozen plasma (FFP) carries the risk of adverse effects such as anaphylactic reactions[6] or transfusion-related acute lung injury (TRALI)[7]. TRALI is one of the most serious and life-threatening adverse reactions, since the incidence of mortality and major morbidity associated with TRALI

is almost as high as that associated with transfusion of ABO-incompatible blood components[8,9]. TRALI has not, however, been reported in association with adminstraiton of solvent/detergent-treated plasma (S/D-plasma)[10]. Another risk of FFP transfusion is transmission of viral diseases such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV)[11]. The solvent/detergent treatment leads to complete removal of all enveloped virus and enhances transfusion safety. Although the rate of HIV, HBV and HCV infections has decreased as a result of more careful donor selection and antibody testing, these viral infections do remain severe and notable complications of transfusion therapy.

Although it is recommended that human plasma is administered as soon as possible after thawing[11], it has been reported that thawed human plasma as been stored for 6 to 24 hours in clinical practice without loss of clotting factor activity[12]. It is assumed that for storage beyond this time, clotting factor activity falls below the minimum standard level recommended for use[13]. This results in wastage of thawed plasma, which increases the economic burden for health care providers[14-16].

There are reports on clotting factor activity in FFP or solvent/detergent-treated plasma (S/D-plasma) during short-term storage (up to 48 hours) [12], or in FFP produced from whole blood after overnight storage[17] and information on a limited number of clotting factors, a limited number of plasma bags or plasma batches, only[18]. Our group has found that the activity of clotting factors is preserved in FFP stored in the short-term[14] and long-term (i.e. 6 days)[15]. However, since little is known about the stability of clotting factors in S/D-plasma, the aim of this study was to investigate clotting factor and inhibitor stability as well as bacterial contamination in S/D-plasma stored at 4 °C±2 °C over a period of 6 days with a focus on clinical applicability according to international quality assurance regulations[19].

Material and methods

Sampling S/D-plasma

The S/D-plasma (Octaplas®) investigated in this study was purchased from Octapharma GmbH, Langenfeld, Germany), we used five different batches for each blood group.

The manufacture and virus inactivation processes of the S/D-plasma used for this investigation have been described elsewhere[20]. In brief, S/D-plasma was produced from pooled plasma of frequent plasma donors. First 380 L of high quality FFP were thawed and underwent a 1 µm filtration to obtain cell-free plasma. This plasma was then treated with 1% tri(n-butyl)phosphate (TNBP) and 1% Triton-X-100 for 4 hours. This was followed by an oil extraction and phase separation to remove the TNBP and the product was filtered again. Hydrophobic interaction chromatography removed the Triton-X-100. Afterwards a third filtration (0.2 µm) was performed. The plasma was then used to fill plasma bags under aseptic conditions and fast-frozen at a temperature below –60 °C and stored below –30 °C. All S/D-plasma was thawed using a plasmatherm III (Barkey, Leopoldshöhe, Germany). Before the samples were drawn, the plasma bags were shaken gently at each sampling time point, just before the samples were taken.

Measurement of clotting factors and inhibitors

After thawing, the S/D-plasma bags were stored at 4 °C for 6 days. The haemostatic parameters measured included fibrinogen, factors II (FII), V (FV), VII (FVII), VIII (FVIII), IX (F IX), X (FX), XI (FXI), XII (FXII) and XIII (FXIII), plasmin inhibitor and von Willebrand factor antigen (VWF-Ag). The clotting inhibitors assayed included antithrombin, protein C and free protein S. Clotting factors and inhibitors were measured imediately after thawing, 6, 24, 48 hours and 6 days after thawing.

The measurement methods and reference ranges were described in the former investigation. Results (except fibrinogen) are given as %-activity of commercially available reference plasma (Instrumentation Laboratory GmbH, Kirchheim, Germany). Reference ranges were obtained from the manufacturers of the test assays.

Blood cultures

A 10 mL sample was taken from each plasma bag after 12, 72 and 144 hours. This 10 mL plasma sample was divided into two aliquots of 5 mL and injected into commercially available bottles for aerobic and anaerobic blood cultures (BD BACTEC, Becton Dickinson, Heidelberg, Germany) and incubated at 35±1.5 °C for 6 days.

Statistical analysis

Given the small sample sizes and/or skewed distributions it was assumed that data were not normally distributed. For this reason, results are given as median and 25% and 75% percentile and only non-parametric tests were performed. Differences between clotting factor and inhibitor activity at the various time points were assessed globally using a non-parametric multivariate analysis of variance (MANOVA) for repeated measurements and small sample sizes with time as the longitudinal measurement. This analysis compared the activity and concentrations of clotting factor and inhibitors from immediately after thawing to 6 hours after thawing (Table I), from 6 to 24 hours after thawing (Table II), from 24 hours to 6 days after thawing (Table III) and from immediately after thawing to 6 days after thawing (Table IV) with regard to all time points simultaneously.

Table I - Clotting factors and inhibitors in S/D-plasma immediately after thawing and 6 hours after thawing.

	Immediately after thawing	6 hours after thawing	p
Fibrinogen mg/dL	280 (263-288)	270 (263-280)	0.2672
FII %	80 (79-82)	82 (80-83)	0.0028
FV %	103 (94-105)	103 (100-107)	0.2577
FVII %	105 (104-109)	106 (103-109)	0.4439
FVIII %	86 (71-89)	78 (71-85)	0.0100
FIX %	104 (99-106)	99 (97-103)	<0.0001
FX %	92 (90-94)	93 (90-93)	0.9639
FXI %	123 (113-138)	121 (111-132)	0.3346
FXII %	96 (93-99)	98 (92-102)	0.2851
FXIII %	90 (89-92)	89 (88-92)	0.2431
Free protein S %	80 (78-83)	82 (80-83)	0.1700
Protein C %	95 (94-97)	94 (93-97)	0.2133
Antithrombin %	95 (93-95)	93 (92-95)	0.1561
Plasmin inhibitor %	33 (28-35)	31 (29-33)	0.4215
VWF-Ag %	135 (105-146)	134 (103-146)	0.2429

Legend: N: 20 plasma samples. The quantities given are medians, results are given in percent of activity, fibrinogen in mg/dL; 25% and 75% confidence intervals are given in brackets after the results.

Table II - Clotting factors and inhibitors in S/D-plasma 6 hours and 24 hours after thawing.

	6 h after thawing	24 h after thawing	p
Fibrinogen mg/dL	270 (263-280)	280 (270-280)	0.3723
FII %	82 (80-83)	78 (77-79)	<0.0001
FV %	103 (100-107)	88 (84-94)	<0.0001
FVII %	106 (103-109)	99 (93-102)	<0.0001
FVIII %	78 (71-85)	76 (64-79)	<0.0029
FIX %	99 (97-103)	98 (96-102)	0.0663
FX %	93 (90-93)	89 (85-92)	0.0229
FXI %	121 (111-132)	118 (101-129)	0.1409
FXII %	98 (92-102)	94 (91-96)	0.1058
FXIII %	89 (88-92)	86 (85-88)	<0.0001
Free protein S %	82 (80-83)	79 (77-81)	<0.0001
Protein C %	94 (93-96)	95 (92-96)	0.3150
Antithrombin %	93 (92-95)	93 (92-95)	0.6752
Plasmin inhibitor %	31 (29-33)	32 (30-34)	0.0192
VWF-Ag %	134 (103-146)	138 (105-145)	0.1678

Legend: N=20 plasma samples. The quantities given are medians, results are given in percent of activity, fibrinogen in mg/dL; 25% and 75% confidence intervals are given in brackets after the results.

Table III - Clotting factors and inhibitors in S/D-plasma 6 and 24 hour after thawing.

	24 h after thawing	6 days after thawing	p
Fibrinogen mg/dL	280 (270-280)	270 (260-270)	0.2384
FII %	78 (77-79)	75 (74-78)	0.0004
FV %	88 (84-94)	88 (84-94)	0.0003
FVII %	99 (93-102)	81 (80-84)	<0.0001
FVIII %	76 (64-79)	70 (63-73)	0.0983
FIX %	98 (96-102)	96 (93-99)	0.7052
FX %	89 (85-92)	92 (90-94)	0.0015
FXI %	118 (101-129)	119 (111-136)	0.3253
FXII %	94 (91-96)	94 (90-98)	0.2056
FXIII %	86 (85-88)	89 (87-90)	0.3616
Free protein S %	79 (77-81)	76 (73-79)	0.0896
Protein C %	95 (92-96)	96 (95-99)	0.2019
Antithrombin %	93 (92-95)	92 (90-94)	0.4367
Plasmin inhibitor %	32 (30-34)	29 (28-31)	0.0019
VWF-Ag %	138 (105-145)	137 (104-147)	0.1988

Legend: N: 20 plasma samples. The quantities given are medians, results are given in percent of activity, fibrinogen in mg/dL; 25% and 75% confidence intervals are given in brackets after the results.

Table IV - Clotting factors and inhibitors in S/D-plasma immediately and 6 days after thawing.

	Immediately after thawing	6 days after thawing	p
Fibrinogen mg/dL	280 (263-288)	270 (260-270)	0.0204
FII %	80 (79-82)	75 (74-78)	<0.0001
FV %	102 (94-105)	88 (84-94)	<0.0001
FVII %	105 (104-109)	81 (80-84)	<0.0001
FVIII %	86 (71-89)	70 (63-73)	<0.0001
FIX %	104 (99-106)	96 (93-99)	<0.0001
FX %	93 (87-101)	92 (90-94)	<0.0001
FXI %	123 (113-138)	119 (111-136)	0.3666
FXII %	96 (93-99)	94 (90-98)	0.3602
FXIII %	90 (89-92)	89 (87-90)	0.0019
Free protein S %	80 (78-83)	76 (73-79)	<0.0001
Protein C %	95 (94-97)	96 (95-99)	0.0371
Antithrombin %	95 (93-95)	92 (90-94)	<0.0001
Plasmin inhibitor %	33 (28-35)	29 (28-31)	0.0299
VWF-Ag %	135 (105-146)	137 (104-147)	0.2205

Legend: N: 20 plasma samples. The quantities given are medians, results are given in percent of activity, fibrinogen in mg/dL; 25% and 75% confidence intervals are given in brackets after the results.

A drop in factor or inhibitor activity of more than 20% or to below international recommendations on quality assurance in plasma production was considered a clinical relevant decrease in plasma quality which would prevent the thawed plasma from being recommended for clinical administration.

Results

From immediately after thawing to 6 hours after thawing

The result of the measurements from immediately after thawing to 6 hours after thawing are shown in Table I. None of the clotting factors or inhibitors,

except FII, FVIII and FIX, showed any significant change over this time period. FII, FVII and free protein S levels increased slightly.

From 6 to 24 hours after thawing

The results from 6 to 24 hours after thawing are summarised in Table II. Slight, but significant decreases were shown for FII, FV, FVII, FVIII, FX, FXIII, free protein S and plasmin inhibitor, whereas fibrinogen, FIX, FXI, FXII, protein C and antithrombin did not show any significant changes in activity.

From 24 hours to 6 days after thawing

The results from 24 hours to 6 days after thawing are displayed in Table III. FII, FV, FX and plasmin inhibitor levels changed significantly. No significant changes were found in any of the other clotting factors.

From immediately after thawing to 6 days after thawing

The results for this period are shown in Table IV. From immediately to 6 days after thawing the activity of clotting factors and inhibitors showed the following patterns: FXI, FXII and VWF-Ag remained stable with no significant loss of activity after 6 days. Fibrinogen, FII, FV, FVII, FVIII as well as FIX, FX, FXIII, protein C, antithrombin, plasmin inhibitor and free protein S decreased significantly over time while protein C and VWF-Ag levels were slightly elevated after 6 days of storage.

Blood cultures

All blood culture bottles were sterile at all time points.

Discussion

There are two main findings of this investigation on the stability of clotting factors and inhibitors in S/D-plasma. First, the activity of all clotting factors and inhibitors, with the exception of FIX, FXI, vWF-Ag, antithrombin and protein C, decreased moderately (below –20%) but significantly during short-term storage from 6 to 24 hours after thawing. After further storage up to 6 days at 4 °C the activity of fibrinogen, FII, FV, FVII, FVIII, FIX, FX, FXIII, PC, AT, plasmin inhibitor and free protein decreased further. Second, the levels of all clotting factors and inhibitors remained stable with low variation above 70% except those of plasmin inhibitor.

The levels of fibrinogen, although stable over 24 hours of storage, decreased significantly (–10 mg/dL) after long-term storage for 6 days. This contrasts with the results of Buchta *et al*.[18] who found that fibrinogen decreased by only 1 mg/dL after 6 days. Given the baseline level of 280 mg/dL, a reduction of 10 mg/dL after 6 days (i.e. 3.6%) could be clinically negligible as the remaining levels may be sufficient to treat moderate factor deficiencies. These results seem to indicate that fibrinogen as the substrate of coagulation is stable during long-term storage of thawed S/D-plasma.

The FII levels in the study by Buchta *et al*.[18] were higher at baseline (1.05 U/mL) but showed similar kinetics during long-term storage (remaining activity after 6 days: 0.98 U/mL, median difference: –7 U/mL). We observed the major decrease from 24 hours to 6 days after storage (–3%) and overall a moderate, though significant, decrease in FII activity from a median 80% to 75% (median difference: –5%) from immediately after thawing to 6 days after thawing. FII should, therefore, be regarded as a stable clotting factor with regard to long-term storage of thawed S/D-plasma. The residual FII activity of 75% might be sufficient to: (i) deliver a sufficient amount of the proenzyme for thrombin generation and (ii) to treat minor prothrombin deficiencies.

The levels of FV and FVII activity decreased significantly to 88% (–14%) and 99% (–7%) over 24 hours of storage and to 88% (–14%) and 81% (–24%, –18% from 24 hours to 6 days), respectively, after 6 days of storage. This shows a rather good stability of FV, while FVII lost its activity mainly in the period from 24 hours to 6 days after thawing. Buchta *et al*.[18] reported that, with the exception of protein S, the activities of all coagulation factors and inhibitors were at least 50% after storage at 4 °C for 6 days. One reason for the different results compared to those of the study by Buchta *et al*.[18] could be the small sample size of only five plasma bags in this latter study[18], whereas in our study five plasma bags from each blood group were studied. In a different study, Nifong *et al*.[21] evaluated 20 S/D-plasma bags, but stored the bags at 20 °C and refroze and rethawed the bags before coagulation tests were performed[21].

Factor activities dropped from 118% to 89% for FV (–29%) and 107% to 87% (–20%) for FVII after the second thawing. Refreezing and rethawing in this study, which is not performed in clinical routine, may account for the differences compared to our results. Given that the decreases in factor activity depended on the duration of storage we conclude that FV can be considered a rather stable clotting factor, while FVII was stable for 24 hours, but not after long-term storage. The preservation of adequate FV level in plasma is of clinical importance, because no FV concentrate is currently available for the treatment of FV deficiency.

The factor of major interest is undoubtedly FVIII because in Europe the level of this factor is the reference parameter for quality assurance with regard to the production of plasma[13]. Our measurements showed decreases of –8% (from 0 to 6 hours), –2% (from 6 to 24 hours), –6% (from 24 hours to 6 days) and –16% (from immediately after thawing to 6 days). Heger *et al*.[12] described a 12% decrease after 6 hours, a 7% decrease from 6 to 24 hours and –22% after 48 hours. These results indicate that FVIII has to be considered a relatively ins clotting factor although factor activity did not approach the lower quality assurance cut off-level after long-term storage at 4 °C.

The median activities of FIX and FX decreased by 8% and 1% over 6 days of storage, which confirms the results from Nifong[21] and Buchta[18]. The change from 24 hours to 6 days was only –2% for FIX and +3% for FX. Although this change of FX was significant, the relative decrease after 6 days was small and the remaining activity was well within the reference range. The same applies to FXI (+1%) and FXII (0%). Regarding the changes from immediately after thawing to 6 days, these were –4% (FXI) and –2% (FXII), which were not statistically significant changes. These clotting factors should, therefore, also be considered as stable clotting factors.

VWF-Ag and FXIII were measured using an antigen assay. Both factors were stable at all time points.

With regards to the inhibitors of the clotting system, there was only a mild decrease in free protein S of –3% from 6 to 24 hours, –4% from 24 hours to 6 days after thawing and –4% after 6 days of storage. Antithrombin decreased by 3% after 6 days, while protein C showed a clinically not relevant but significant increase over the entire observation period. In comparison, earlier studies found a decline of protein S during storage at 4 °C from 0.41 U/L to 0.18 U/L (0.33U/L after 24 hours) for S/D-plasma and relative stability of antithrombin (1.05 U/mL to 0.95 U/mL, median difference: –10 U/mL) and protein C (0.94 U/mL to 0.95 U/mL, median difference: +0.1 U/mL)[18]. These results may be more prone to the influence of outliers because of the small number of plasma bags investigated (n=5). Furthermore it should be noted that baseline activity of protein S was remarkably lower than in our investigation. This might be due to different measurement methods. It is notable that the initial value of protein S was about 50% lower and declined to a half. Consistent with results of the literature antithrombin and protein C should be considered stable inhibitors with regard to long-term storage of thawed plasma for 6 days. Although the results for free protein S are conflicting, the relative instability of this inhibitor is supported by the results of a study by Heger *et al.*[12], who showed a significant decrease (–21%) after storage for 24 hours at 4 °C[12]. These results, together with our own, indicate a relative stability of protein S over 24 hours. The activity of clotting inhibitors may be important in clinical conditions characterised by an activation of the coagulation system (e.g. disseminated intravascular coagulation)[22], when replacement of inhibitor activity is required. However, it must be mentioned that free protein S was measured by an antigen assay, so conclusions cannot be made on its activity.

It is well known that the activity of plasmin inhibitor is influenced by the solvent-detergent virus inactivation process. Our results showed a low activity of the inhibitor after thawing (33%) compared to the level of activity in FFP[12]. Plasmin inhibitor decreased by 1% after 24 hours and by 3% from 24 hours to 6 days. Although the activity of plasmin inhibitor in thawed S/D-plasma is low compared to that in FFP, the kinetics of plasmin inhibitor in the S/D-treated plasma are consistent with the results of Buchta *et al*.[18] and Beeck *et al*.[23] and suggest that plasmin inhibitor should be considered a stable antifibrinolytic protein in thawed S/D-plasma although its activity is low and thawed S/D plasma is not, therefore, suitable for treating conditions with elevated fibrinolytic activity.

Transfusion-related transmission of bacteria is another issue in the discussion about the clinical

suitability of stored, thawed plasma. We found no bacterial contamination during long-term storage of S/D-plasma. Sterility has been reported for storage at 20 °C for 24 hours[21] and 4 °C for 6 days[13]. The lack of contamination is remarkable as daily samples were taken from plasma bags, which would not occur in clinical practice. However, further investigations on bacterial contamination of stored plasma are warranted, as only 20 units of S/D-plasma were evaluated in our study.

Regarding the question of whether factor activity in thawed S/D-plasma remains high enough for clinical usage, the published data on the stability of coagulation factors were gained from measuring very few batches[12,18] and should, therefore, be interpreted with caution. Although most clotting factors showed no further significant changes from 24 hours to 6 days after thawing, storage beyond 24 hours was associated with major decreases in two of the important factors for bleeding patients, FVII and FVIII, thereby impairing the quality of S/D-plasma. For clinical usage we conservatively conclude that short-term storage of thawed S/D plasma for up to 24 hours appears acceptable as levels of haemostatic factors, except plasmin inhibitor, remain reliably within safe ranges.

For departments requiring high volumes of blood and plasma products, these data support the possibility of establishing an emergency reserve of thawed plasma to facilitate rapid administration of plasma. In particular, military hospitals could profit from this as patients with combat injuries have a high risk of death from exsanguination and coagulopathy is an independent predictor of mortality[24] and still represents the cause of death in about 40% of trauma patients[25]. Recent results regarding the treatment of bleeding trauma patients have shown that the early administration of large volumes of plasma decreased mortality[26]. However, the low plasmin inhibitor levels in S/D-plasma prevent the use of the blood product in conditions with high fibrinolytic activity. High fibrinolytic activity contributes to trauma-associated coagulopathy[27]. In these patients S/D-plasma with low plasmin inhibitor levels may not be efficacious as FFP and/or coagulation factor concentrates[28] and will require the concomitant use of antifibrinolytics[29].

Apart from faster availability, our results show that wastage may be prevented in the case that already thawed plasma is not transfused. Downes *et al*. reported that the extension of the expiry time of FFP from 24 hours to 3 days resulted in a reduction of wastage by 78% and cost savings of 17,800 $ per year[16].

Conclusions

Our data indicate that, from an *in vitro* point of view, clotting factors remain stable in thawed S/D-plasma stored for 24 hours at 4 °C. Longer storage of plasma for up to 6 days results in limited quality. These results may improve availability of plasma in emergency situations and reduce hospital costs by decreasing wastage of plasma.

Conflicts of interest disclosure

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