Fresh frozen plasma is ineffective for reversal of coagulation factor dilution in an in vitro model

C. Jámbor[1,2], V. Lesch[1], T. W. Schnider[1,3], G. Kreienbühl[1], W. Korte[2,3]

[1]Institute for Anaesthesiology and [2]Institute for Clinical Chemistry and Hematology, Kantonsspital St. Gallen, Switzerland; [3]University of Bern, Switzerland

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Abstract

As fresh frozen plasma (FFP) loses coagulation factor activity during the production process, FFP therapy may not be feasible to completely replenish coagulation factor concentrations in a clinical setting. The aim of this study was to describe the results of early replacement coagulation factors with FFP in a simplified and idealized in vitro model of dilution. Six ml of whole blood drawn from healthy volunteers was first diluted. Imitating blood loss and replacement with FFP thereafter, 20%, 40%, 60% and 80% of the diluted whole blood was removed and replaced with FFP. Levels of coagulation factors were analyzed with standard assays. FFP did not result in replenishing of coagulation factors. Rather, a trend to a further decrease of most coagulation factors was seen. In this in vitro study, a modelled, limited dilution was stabilized but not reversed by replacing (imitated) blood loss with FFP. As continued coagulation factor consumption and additional dilution due to volume replacement occur in the clinical setting, our data suggest that the use of coagulation factor concentrates seem necessary to replenish coagulation factor activities.

Introduction

Intraoperative dilutional coagulopathy is a common and frequent clinical problem. Establishing normovolaemia with crystalloids or colloids during blood loss, results in normovolaemic dilution of coagulation factors and to a consecutive dilutional coagulopathy. Continued blood loss and consumption of coagulation factors deteriorate an existing coagulopathy *in vivo*, rendering an adequate estimation of the remaining coagulation factor concentrations essentially impossible.

Lundsgaard-Hansen suggested that 20% of the whole blood volume can be exchanged without transfusing packed red cells, 90% without transfusing coagulation factors and 140% before platelets reach critically low levels (1). As blood loss and dilutional coagulopathy can often be anticipated to some extent, it would be helpful to have a prospective approach at hand that allows determination of the required blood components and volumes in order to prevent continued coagulopathy. Mortelmans developed a simple method for calculating component dilution during isovolemic fluid resuscitation (2). This mathematical model describes the concentration course of blood components remaining within the intravascular space, such as coagulation factors during isovolemic component dilution.

How to maintain coagulation factors at concentrations that allow adequate local hemostasis during major hemorrhage with the concomitant need for ongoing fluid resuscitation is still a matter of debate. Coagulopathy is frequently encountered during resuscitation for major hemorrhage, which is still a major cause of death in trauma patients (3). This suggests that current coagulation factor replacement strategies can probably be improved. Ho et al. recently developed a pharmacokinetic model to simulate the dilutional part of coagulopathy during hemorrhage and compared different

FFP transfusion strategies for the prevention and correction of dilutional coagulopathy (4). In this study, the authors calculated that the equivalent of a whole-blood transfusion is necessary to correct or prevent dilutional coagulopathy during resuscitation of a patient with major trauma.

The aim of this study was to evaluate the influence of early FFP transfusion on coagulation factor concentrations using an idealized and simple *in vitro* model of mild dilution. We hypothesized that early administration of FFP sufficiently reverses the dilution of coagulation factors.

Methods

Following approval by the local ethics committee, four healthy male volunteers gave written informed consent and were included in the study. Exclusion criteria were consumption of any medication within the previous ten days or any kind of a bleeding history.

Thirty-six ml of blood were drawn from each volunteer from an antecubital vein without tourniquet using an 18-Gauge needle. The blood was collected into six blood collection tubes of six ml (Vacutainer®, BD, Basel, Switzerland) containing 0.105 M sodium citrate as anticoagulant. One tube was kept undiluted for control („Un"). To the six ml whole blood in the remaining five tubes, 2 ml of Ringer Lactate (RL) solution was added, creating a mild dilution as a baseline ("B"). To imitate blood loss and replacement with FFP to a varying extent, 20%, 40%, 60% or 80% of the diluted whole blood (one sample each) was removed and replaced with FFP of AB blood group, respectively. Thus, we established the following series of citrated blood: undiluted sample („Un"); primary dilution with RL („B"); diluted samples of which 20%, 40% , 60% or 80% of the volume was replaced with FFP, respectively ("20%", "40%", "60%", "80%"). After preparation, samples were centrifuged for 20 minutes at 3000G and 22 °C. Supernatant plasma was aliquoted and frozen at -80 °C until further analysis.

Fibrinogen was determined with the Clauss method; FII, FV, FVII, FVIIIc and FX, were determined by single step clotting assays using factor deficient plasma; FXIII, AT and Protein C were determined with chromogenic assays. All assays were performed on a BCS analyser (Siemens Diagnostics, Dade Behring, Marburg, Germany).

Single donor fresh frozen plasma (blood group AB) was obtained from the local transfusion service. Blood drawing from volunteers was sequential. Therefore, the fresh frozen plasma had been aliquoted and was kept at -80° Celsius until further use. Factor VIII activity of FFP was determined before and after storage to control for potential loss.

Statistical analysis was performed with Sigma Stat Version 3.11 (Jandel, San Rafael, CA, USA). Repeated measurements ANOVA with posthoc Bonferroni's correction were performed to detect differences between series. Results are described as mean and standard error (SE). Statistical significance was set to $p \leq 0.05$.

Results

Activity of coagulation factors decreased significantly after dilution with Ringer Lactate, indicating a dilution associated loss of coagulation factor activities. Using FFP to replace a further, imitated blood loss did not improve this dilution-induced decrease of coagulation factor activities; rather, a further non-significant decrease of coagulation factor activities was observed (Table 1, Fig. 1a-b). This was observed for activities of fibrinogen and factors II, V, VII, VIII and protein C. Factor XIII and antithrombin behaved somewhat differently. Replacement of very high volumes of FFP (here 40 to 80% of the imitated whole blood volume) resulted in increasing FXIII activity after intial dilution; a similar, but less pronounced trend was seen for antithrombin activity (Table 1, Fig 1c). The only factor which was not significantly diluted after using Ringer Lactate was factor X; this factor never showed significant variation from baseline (Table 1, Fig. 1b).

Factor VIII activity of the FFP used for replacement was 62% before and 50% after storage.

Discussion

The main finding of this study was that dilution-induced decrease of coagulation factors can be stabilized but not reversed by early replacement of even high proportions of FFP in this in vitro model.

However, as the results obtained for the different coagulation factors are not homogeneous, a differentiated interpretation is necessary. With FFP, the procoagulants fibrinogen and factors II, V, VII, VIII and the inhibitors protein C and antithrombin were kept on the lower level observed after primary dilution with Ringer Lactate.

Table 1. Coagulation factor activities at baseline (B), 20%, 40%, 60% and 80% of volume exchanged with FFP as compared to undiluted sample (Un).

	Un	**B**	**20 %**	**40 %**	**60 %**	**80 %**
Fibrinogen (g/l)	2.9(0.3)	1.7(0.2)	1.8(0.2)	1.7(0.1)	1.6(0.1)	1.5(0.2)
F II (%)	104(8.7)	68(8.0)	76(94.8)	71(3.0)	68(4.0)	65(6.4)
F V (%)	102(8.7)	72(20.4)	80(8.0)ns	74(5.1)	66(3.3)	66(7.3)
F VII (%)	107(12.8)	77(11.8)	81(10.2)ns	79(3.5)	75(5.8)	69(5.8)
F VIIIc (%)	84(9.6)	60(10.7)	62(8.8)	55(5.0)	54(5.0)	48(6.9)
F X (%)	73(7.7)	60(7.8)ns	66(5.4)ns	62(1.1)ns	64(2.5)ns	60(6.4)ns
F XIII (%)	104(10.7)	65(6.2)	80(7.0)	86(6.4)ns	90(7.1)ns	91(7.9)ns
AT (%)	96(3.1)	58(4.6)	69(2.3)	71(2.0)	73(4.2)	73(5.8)
PC (%)	115(13.9)	75(10.1)	78(7.8)	75(3.1)	70(4.1)	68(5.7)

data are depicted as the mean (standard error).
ns: non significant vs. undiluted, all other values are significantly different as compared to undiluted

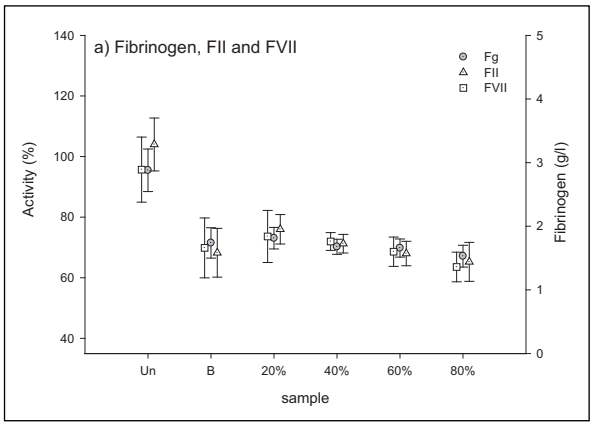

Figure 1a. Activity of fibrinogen and factor II and VII in unmanipulated sample (Un), after baseline dilution (B) and replacement of 20%, 40%, 60% or 80% of the diluted whole blood with FFP (20%, 40%, 60% and 80%, respectively). Error bars show standard error (SE).

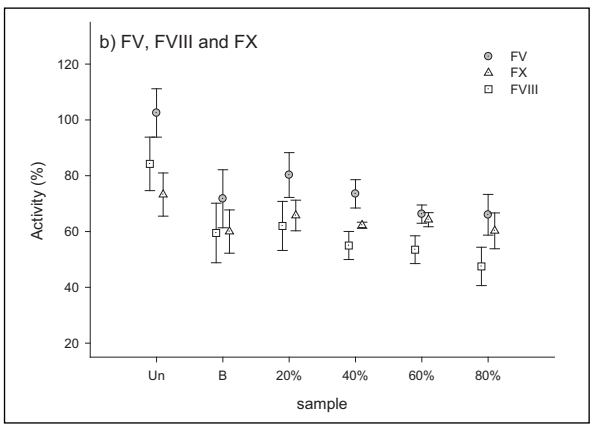

Figure 1b. Activity of factor V, VIII and X

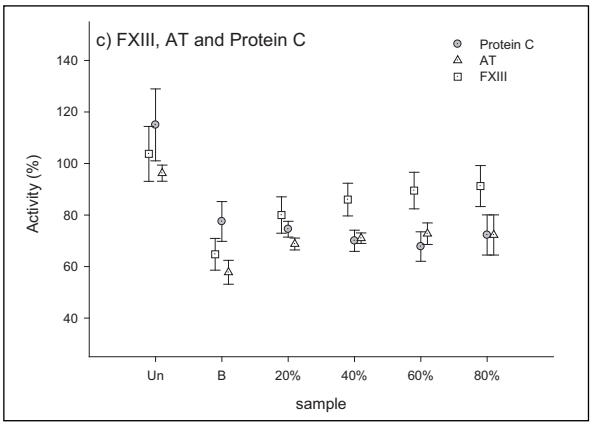

Figure 1c. Activity of factor XIII, antithrombin and protein C

Factor X did not show a significant reduction after initial dilution with Ringer Lactate. This is in contrast to animal (rabbit) studies (5); the differences, however, seem explainable: in that study, the replaced volume was larger, which is a likely explanation for the differences observed; in addition, a consumptive effect might have occurred; and factor levels in the rabbit are higher to begin with, suggesting a bigger relative effect when dilution is performed.

An interesting observation within our study is the fact that, while dilution conditions were identical with all coagulation factors, factor X did not show a significant decrease, whereas all other factors did. Factor X activation (and thus results of the assays used here) is dependent on colloid osmotic pressure as well as salt concentration which might explain the results observed at least to some extent.

Factor XIII was the only factor that showed replenishment after initial dilution, but only with very high volumes of FFP replacement (40 – 80%). If these results were to be translated into clinical practice, the volume needed to provide adequate factor XIII replenishment (extrapolation from our data would suggest the need of at least 2 liters of FFP) would probably prohibit its use for this particular indication.

One additional, important issue has to be recognized with all these considerations: our *in vitro* model does not comprise further *in vivo* dilution and consumption of coagulation factors. Additional dilution and consumption, however, occur in daily practice during blood loss. Therefore, our idealized model represents the best case scenario. Thus, we assume that the use of FFP may not lead to the reversal of a dilution in vivo. Especially in case of high volumes of blood loss, if a clinically relevant dilutional coagulopathy exists, the sole use of FFP might not result in increase of coagulation factor activities.

There are some limitations of this study. First, using an *in vitro* model, the results obtained can not directly be translated into clinical practice. As the model used in our study represents the best case clinical scenario however, cautious interpreting of the data concerning the clinical impact of FFP transfusion is justifiable. Second, the study is somewhat underpowered, making interpretation of the data difficult as the individual data are highly variable. Although the study may benefit from additional study samples, the small sample size used in our study was sufficient to demonstrate significant differences. As the aim of the study was to describe the influence of *in vitro* FFP transfusion on coagulation factor concentrations in prepared blood samples obtained from healthy volunteers, additional study samples probably would not have altered the results. Third, to guarantee that the blood of all volunteers was processed with the same FFP, quarantine FFP was aliquoted into four satellite bags and frozen at -80 °C. This resulted in a 12% loss of factor VIII activity, which is the factor most sensitive to loss due to freezing procedures (6). However, all factors evaluated showed normal levels in the material used for replacement experiments (65-111%, fibrinogen 2.3 g/l). This is in line with observations by others who have found that vitamin K-dependent coagulation factor and fibrinogen concentrations remain stable and adequate for transfusion even in twice-thawed FFP (7).

In conclusion, results of this in vitro dilution model, using idealized conditions suggest that replacement of cogulation factors with FFP after initial dilution and further blood loss will not result in an increase of coagulation factor activity. Despite limitations, this in vitro study has potential clinical implications in using FFP as a replacement therapy in major blood losses. In daily practice a continued – albeit slower – decrease in coagulation factor may result. Before any firm conclusions for daily practice can be drawn, controlled clinical trials to evaluate this question are necessary.

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Address for corresponding: Csilla Jámbor, M.D., Clinic for Anesthesiology, University of Munich, Max-Lebsche-Platz 32, 81377 Munich, Germany, E-Mail: csilla.jambor@web.de