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Influence of prothrombin complex concentrates on plasma coagulation in critically ill patients

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Abstract *Objective:* To evaluate thrombogenicity of prothrombin complex concentrates (PCCs) in critically ill patients.
Design: Prospective clinical study.
Setting: Medical intensive care unit at a university hospital.
Patients: 16 consecutive patients suffering from acquired deficiencies of coagulation factors and with either overt bleeding from any site or a planned invasive procedure.
Interventions: 2000 factor IX units of PCCs intravenously.
Measurements and results: Prothrombin time (PT), activated partial prothrombin time, fibrinogen, platelet count, plasma levels of coagulation factors II, V, VII, VIII, IX, X, antithrombin, protein C, thrombin-antithrombin complex (TAT), prothrombin fragment F_{1+2}, and the fibrin degradation product D-dimer were measured prior to and 1, 3, and 24 h after administration of PCCs. PT as well as coagulation factors II, VII, IX, and X, TAT, and F_{1+2} showed a significant increase after administration of PCCs. All other parameters remained unchanged.
Conclusions: Administration of PCCs induces thrombin generation. No evidence for induction of disseminated intravascular coagulation in biochemical terms could be found. When rapid correction of acquired coagulation factor disturbances is warranted, the use of PCCs seems reasonable, but the elevated risk of intravascular thrombus formation should be kept in mind.

Key words Prothrombin complex concentrates · Thrombogenicity · Intensive care · Disseminated intravascular coagulation

Introduction

Prothrombin complex concentrates (PCCs) containing coagulation factors II, IX, and X, with or without factor VII, have been used for years in the treatment of inherited coagulation factor deficiencies, particularly in factor IX deficiency [1, 2]. Furthermore, PCCs have been proposed as a substitution therapy in acquired coagulation factor deficiencies or as antagonists to warfarin-like anticoagulants [3–5]. With the widespread use of PCCs, an increasing amount of data on possible side effects has been published: the association with thromboembolic complications [6–8] and activation of the coagulation cascade [9–11] have led to a call for cautious use of PCCs. A survey of thromboembolic episodes during treatment with PCCs conducted by Jeanne Lusher on behalf of the International Society on Thrombosis and Hemostasis reported 72 cases between 1987 and 1990 [6]. Most of the data concerning thrombogenicity and activation of the coagulation cascade were based on laboratory experiments [12–14] or on small-scale clinical studies in hemophilia B patients [15–18]. Substitution of coagulation factors is a major concern in the intensive care setting. Critically ill patients frequently undergo invasive procedures while suffering from disturbances of plasma coagulation and are therefore at a higher risk of bleeding. Administration of PCCs seems more convenient than transfusion of fresh frozen plasma with regard

Table 1 Patient characteristics. Values are number of patients and mean ± SD (*ARDS* adult respiratory distress syndrome, *CPR* cardiopulmonary resuscitation)

Diagnoses	
Peritonitis	1
CPR	3
Sepsis	2
Hepatic failure	4
Major thoracic surgery	1
ARDS	1
Pneumonia	4
Indication for substitution	
Gastrointestinal bleeding	4
Postoperative bleeding	4
Dialysis catheter implantation	5
Transbronchial biopsy	2
Intracranial bleeding	1
Baseline characteristics	
Age (years)	48 ± 16
Body weight (kg)	72 ± 44
APACHE III admission score	46 ± 28
Fibrinogen (mg/dl)	399 ± 236
Platelet count ($\times 10^9$/l)	156 ± 116
Hemoglobin (g/dl)	9.5 ± 1.9
Albumin (g/l)	27 ± 8
Bilirubin (mg/dl)	2.7 ± 2.8

to volume overload and speed of administration. On the other hand, the fear of inducing thrombotic complications or consumption coagulopathy has led many intensivists to adopt a cautious attitude toward substitution of coagulation factors. In vivo data on the use of modern PCC preparations in patients suffering from acquired coagulation factor deficiencies are limited; prospective studies investigating the effects of PCCs on coagulation activation in critically ill patients especially are missing. We therefore conducted a prospective study on the influence of PCCs on coagulation parameters in critically ill patients suffering from coagulation factor deficiency due to inadequate hepatic synthesis, in whom overt bleeding or the necessity of invasive procedures made substitution of coagulation factors necessary.

Patients and methods

The study was performed between November 1997 and June 1998 according to the guidelines of the local ethical review board. Patients were eligible if the prothrombin time, expressed as a percentage of normal, was < 50 % of a normal plasma pool (thromboplastin time) and bleeding from any site was present or an invasive diagnostic or therapeutic procedure involving the risk of bleeding, i. e., implantation of intravascular catheters or a biopsy, was planned. The exclusion criteria were planned surgery during the study period, heparin therapy, and clinically overt disseminated intravascular coagulation (DIC). Since molecular markers to diagnose DIC rapidly are not part of the hospital routine yet, we chose a definition of DIC based on clinical judgment and routine laboratory parameters for inclusion: presence of a clinically apparent thrombohemorrhagic disorder and a decrease in platelet count of more than 40×10^9/l and/or fibrinogen of more than 50 mg/dl within 24 h.

Sixteen consecutive patients admitted to the intensive care unit (10 males, 6 females, median age 57 years, range 23–67 years) were enrolled in the study. Baseline laboratory data were collected from routine laboratory testing on the day of inclusion and Acute Physiology and Chronic Health Evaluation III scores were calculated after admission to the intensive care unit by using the worst available values during the first 24 h in the unit according to Knaus et al. [19]. Hemoglobin levels were kept above 8.0 g/dl by transfusion of packed red blood cells. None of the patients received heparin, derivatives from human blood plasma, or inhibitors of fibrinolysis, like aprotinin, during the study period. Additional therapy consisted of mechanical ventilation in 14 patients, substitution of crystalloid fluid in all patients, broad-spectrum antibiotic therapy in 12 patients, and vasopressor therapy in 5 patients. None of the patients underwent extracorporeal circulation during the study. Patients' characteristics and baseline laboratory findings are given in Table 1.

First, 2000 factor IX units (i. e., four packages) of a commercially available PCC (Beriplex P/N, Centeon, Marburg, Germany) were reconstituted from the lyophilisate according to the manufacturer's guidelines. In this preparation, virus elimination is provided by pasteurization and nanofiltration [20]. According to the manufacturer, a package contains 640 IU of coagulation factor II, 340 IU of factor VII, 500 IU of factor IX, 760 IU of factor X, 600 IU of protein C, 8–40 IU of heparin, 4–30 IU of antithrombin, 40–80 mg of human albumin as well as sodium chloride and sodium citrate. The preparation was administered over 60 min via a perfusor syringe. Blood samples were drawn prior to administration, immediately afterwards, and 3 and 24 h later. All blood samples were drawn from separate venous puncture sites. For coagulation tests, blood was collected into trisodium citrate, for platelet counting into ethylenediaminetetrabracetic acid. Blood was centrifuged immediately after being drawn at 3000 U/min for 30 min at 4 °C and then stored at −70 °C until work-up. The following parameters were assessed at all time points: prothrombin time (PT), partial prothrombin time (aPTT), fibrinogen, platelet count, plasma levels of coagulation factors II, V, VII, VIII, IX, X, antithrombin, protein C, thrombin-antithrombin complex (TAT), prothrombin fragment F_{1+2} (F_{1+2}), and D-dimer. PT (Normotest, Nycomed, Oslo, Norway) and aPTT (Pathromtin, Behring, Marburg, Germany) were measured using a KC-10 coagulometer (Amelung, Lieme, Germany). The patient sensitivity index of the thromboplastin used for PT determination was 0.89, the correction factor was 0.10. Coagulation factors II, V, VII, VIII, IX, and X were measured using factor-deficient plasmas [21] (Behring, Marburg, Germany for coagulation factors II, V, VII, and X; Immuno, Vienna, Austria for coagulation factors VIII and IX). Antithrombin levels were determined by a chromogenic method (Berichrom, Behring, Marburg, Germany), protein C levels were assessed by enzyme-linked immunosorbent assay (ELISA) (Asserachrom Protein C, Roche, Vienna, Austria). TAT, F_{1+2}, and D-dimer were assessed by ELISA using commercially available kits (Behring, Marburg, Germany for TAT and F_{1+2}, and Boehringer, Mannheim, for D-dimer). Fibrinogen was assessed according to the Clauss method, platelets were counted in a counting chamber. To correct for the dilution of plasma by citrate, the values obtained for plasma protein activities were recalculated after correction for the hematocrit according to the following formula proposed by Seligsohn et al. [22]:

$$\text{Correction factor} = (\text{hematocrit} + 11.1)/\text{hematocrit}.$$

All data are shown as mean ± SD except when indicated otherwise. Difference over time course and between single time points were

Table 2 Laboratory findings. (Values are mean ± SD. Values in parentheses are hematocrit corrected (*PT* prothrombin time, *aPTT* partial thromboplastin time, *Hb* hemoglobin, *AT* antithrombin, *F* coagulation factor, *TAT* thrombin-antithrombin complex, F_{1+2} prothrombin fragment F_{1+2})

	Time point				Sig.[a]
	0	1	3	24	
PT (s)	35 ± 8	26 ± 5[b]	27 ± 5[b]	29 ± 6	$p < 0.0001$
aPTT (s)	44 ± 7	38 ± 7	46 ± 7	37 ± 8	NS
Platelets (× 10^9/l)	156 ± 116	154 ± 104	149 ± 116	161 ± 124	NS
Hb (g/dl)	9.5 ± 1.9	9.1 ± 2.3	9.6 ± 1.5	9.4 ± 1.6	NS
Fibrinogen (mg/dl)	399 ± 236 (550 ± 325)	411 ± 256 (580 ± 361)	414 ± 224 (567 ± 307)	421 ± 240 (581 ± 331)	NS
AT (%)	57 ± 36 (80 ± 51)	57 ± 32 (82 ± 52)	56 ± 40 (80 ± 54)	54 ± 44 (68 ± 44)	NS
Protein C (%)	36 ± 13 (50 ± 19)	77 ± 24[b] (110 ± 42)[b]	65 ± 17[b] (88 ± 24)[b]	41 ± 13 (56 ± 18)	$p < 0.0001$
F II (%)	32 ± 8 (45 ± 14)	61 ± 20[b] (86 ± 28)[b]	54 ± 16[b] (74 ± 24)[b]	44 ± 12 (65 ± 25)	$p < 0.0001$
FV (%)	14 ± 12 (20 ± 18)	15 ± 12 (21 ± 20)	12 ± 8 (17 ± 14)	10 ± 4 (18 ± 11)	NS
F VII (%)	32 ± 48 (44 ± 63)	43 ± 32[b] (60 ± 42)[b]	40 ± 36 (54 ± 46)	19 ± 12 (45 ± 39)	$p < 0.001$
F VIII (%)	81 ± 40 (112 ± 56)	94 ± 44 (132 ± 58)	81 ± 40 (110 ± 55)	83 ± 64 (111 ± 65)	NS
F IX (%)	60 ± 52 (83 ± 74)	87 ± 60[b] (125 ± 86)[b]	73 ± 44 (99 ± 61)	68 ± 44 (93 ± 42)	$p < 0.001$
F X (%)	34 ± 12 (47 ± 21)	72 ± 21[b] (103 ± 40)[b]	63 ± 20[b] (86 ± 29)[b]	51 ± 24[b] (78 ± 29)[b]	$p < 0.0001$
TAT (ng/ml)	19.4 ± 18.0 (26 ± 24)	28.2 ± 21.6[b] (39 ± 29)[b]	29.2 ± 36.8 (40 ± 50)	24.8 ± 36.4 (34 ± 50)	$p < 0.001$
F_{1+2} (nmol/l)	2.5 ± 1.6 (3.4 ± 2.3)	10.3 ± 6.4[b] (14.3 ± 8.3)[b]	6.3 ± 4.8[b] (8.5 ± 6.6)[b]	3.8 ± 1.6 (5.3 ± 3.3)	$p < 0.0001$
D-dimer (µg/ml)	5.9 ± 7.6 (8.1 ± 10.5)	6.3 ± 8.8 (8.9 ± 12.2)	6.6 ± 10.0 (9.1 ± 14.2)	8.9 ± 10.0 (12.3 ± 14.3)	NS

[a] *p* values denote statistically significant differences over time course calculated by Friedman ANOVA
[b] Statistically significant differences compared to baseline

calculated by a one-way nonparametric repeated measures test (Friedman analysis of variance). A *p* value < 0.05 was regarded as statistically significant.

Results

All patients completed the study. Bleeding, indicated by hemoglobin levels, ceased in all cases. No bleeding complications occurred during any of the invasive procedures. No further substitution of human blood plasma derivatives, coagulation factors, or inhibitors of fibrinolysis like aprotinin was necessary during the study period. Three patients received 2 units of packed red blood cells each. In none of the patients were clinically overt thromboembolic events observed. The laboratory findings are listed in Table 2. PT, protein C, as well as coagulation factors II, VII, IX, and X, showed a significant increase after administration of PCCs. Moreover, TAT and F_{1+2} increased significantly after administration of PCCs. All of these parameters decreased toward baseline within 24 h. aPTT, fibrinogen, platelet count, hemo-

globin levels, antithrombin levels, coagulation factors V and VIII, and d-Dimer levels did not change significantly. The statistical results remained unchanged after correction for plasma dilution (Table 2).

Discussion

In our study, a dose of 2000 factor IX units of a PCC (mean of 30 U/kg body weight) was sufficient to normalize PT by raising the plasma levels of coagulation factors II, VII, IX, and X in patients with moderately reduced coagulation activity. We used a fixed dose of 2000 factor IX units as a very practical therapeutic approach. Based upon the calculations provided by the manufacturer, a dose of 1 IU/kg body weight of PCCs should raise the PT by 1% in terms of percent of normal. The PT decreased from a mean of 35 s to 26 s, i.e., an increase from 46 to 77%. The expected mean increase in our patients with a mean weight of 72 kg would have been 28%, the measured mean increase was 31%. Thus, the increase in PT very closely approached the ex-

pected one. It is interesting that based on these calculations, no reduced bioavailability of the exogenously applied coagulation factors was observed despite the presence of moderate hepatic failure in many of our patients. To achieve similar effects with fresh frozen plasma, administration of up to 2500 ml or even more would have been necessary based on the same calculations. A volume load to that extent could be deleterious in patients suffering from cardiac failure. In contrast, preparation and administration of PCCs is easy and can be performed rapidly.

The effect of normalizing plasma coagulation, as indicated by PT, was still observed after 3 h and values had returned close to baseline within 24 h. The finding that a "time window" of more than 3 h after administration of PCC allows interventions under the condition of a normalized plasma coagulation seems to be important to clinicians for scheduling invasive procedures. Interestingly, aPTT, which was marginally elevated at baseline, normalized after infusion of PCCs but returned to baseline within 3 h. The kinetics of factors VIII and IX do not explain this observation; ongoing fibrinolysis can be excluded by stable levels of D-dimer. As the time course of aPTT did not reach statistical significance, the observed differences might as well have occurred by chance.

Our data on the correction of abnormal laboratory parameters suggest that the substitution of coagulation factors in patients suffering from acquired coagulation factor deficiencies seems to be an easy and effective way in emergency situations. However, both venous and arterial thromboembolic events have been attributed to the use of PCCs [6, 8, 23–26]. Moreover, induction of DIC was associated with administration of PCCs [11]. The exact causes of thrombogenicity associated with PCC therapy are not completely understood. Concentrates may contain activated coagulation factors and coagulant-active phospholipids, which might lead to an imbalance between procoagulant and anticoagulant pathways of hemostasis in the plasma [28–31]. Repeated high doses of PCCs could lead to supranormal values of not deficient coagulation factors, thus contributing to a hypercoagulable state [29, 32]. Modern PCC preparations like the one used in our study contain only small amounts of activated coagulation factors, especially factors VIIa and IXa, which seem to be important mediators of PCC-associated thrombogenicity [16, 33]. In a comparison of various PCC preparations, the one used in our study was found to contain relatively small amounts of activated factor VII [34]. No thromboembolic episodes were observed in our patient population; however, our study had far too few patients and was not designed to assess clinical endpoints as the incidence of thromboembolic complications. Most complications were reported in association with repeated high doses of PCCs and concomitant risk factors like

major surgery or crash injuries, and the use of aprotinin [35–37].

With respect to laboratory findings on activation of plasma coagulation, we observed a significant increase in F_{1+2} and TAT. F_{1+2} is a measure of the cleavage of prothrombin by activated factor X being released from the amino-terminal portion of the molecule during its conversion to thrombin. Elevated levels, therefore, indicate intravascular thrombin formation. The same is true for TAT, which is formed by inhibition of thrombin by antithrombin in the case of thrombin formation [38]. Although the elevated levels of F_{1+2} after infusion of PCCs were in part attributed to a certain amount of F_{1+2} present in reconstituted PCCs [18], the amount of the increase observed in our study and the concomitant increase in TAT levels make the exogenous administration of a large amount of F_{1+2} unlikely [17]. The observed increase in both TAT and F_{1+2} thus indicates in vivo thrombin generation and the presence of a prethrombotic state. A prethrombotic state can be defined as an imbalance in hemostasis with a tendency to hypercoagulability due to an activation of enzymes of the coagulation cascade without clinical signs of thrombosis or laboratory evidence of fibrin deposits [39, 40]. In biochemical terms, an increase in the enzymatic activity of factor Xa in thrombin formation (reflected by the elevation of F_{1+2} and TAT levels) defines a prethrombotic state [40].

Since the coagulation cascade obviously was activated by infusion of PCCs, does this resemble DIC? According to the definition of DIC as a dynamic process involving thrombin and fibrin formation, DIC consists of (1) consumption coagulopathy leading to depletion of coagulation factors enhancing the risk of bleeding complications, (2) fibrinolysis, which could further aggravate bleeding, and, on the other hand, (3) hypercoagulopathy [41, 42]. Consumption coagulopathy and fibrinolysis may be present to different extents and do not necessarily parallel hypercoagulopathy. Thus, hypercoagulopathy without evidence of coagulation factor consumption and/or fibrinolysis may be a prodromal state of DIC, but in many cases resembles a prethrombotic or hypercoagulable state [41]. In our patients, a short peak of elevated markers indicating activation of the coagulation system leading to thrombin formation was observed, but neither prolonged consumption of thrombin nor a parallel increase in fibrinolytic activity could be found. D-dimer, a neoantigen formed as the result of plasmin digestion of cross-linked fibrin [43], had increased, though not significantly, after 24 h but did not change during the hours following the peak of TAT and F_{1+2}. Moreover, neither consumption of an inhibitor (antithrombin) nor consumption of not substituted coagulation factors or platelets could be observed. Therefore, our results resemble the definition of a prethrombotic state rather than DIC, and we cannot derive induction

of DIC by PCC infusion from our data. These findings are consistent with the results of previous work comparing the thrombogenicity of PCCs with highly purified factor IX concentrates in hemophilia B patients [15, 17, 18].

Prophylactic administration of antithrombin has been proposed to avoid thromboembolic complications after administration of PCCs [37]. This approach, however, cannot yet be substantiated by prospectively acquired data. Since antithrombin has not been shown to exert clear-cut positive effects in other disease entities like DIC or perioperative prophylaxis of thrombosis [44], we do not think that it can be recommended yet as standard therapy.

Modern PCCs contain protein C in various amounts – in our preparation, 600 IU per package – thus patients received 2400 IU of protein C. Protein C is a natural anticoagulant and has been found to exert positive effects in patients suffering from severe consumptive coagulopathies, especially in DIC induced by meningococcal infections [45, 46]. The dose administered in those studies was considerably higher than that given to our patients, nevertheless protein C levels were raised to normal or supranormal levels in nearly all patients. We cannot tell from our data to what extent protein C as a component of PCCs can prevent thromboembolic complications. Although most reported complications with the use of PCCs occurred with the older preparations containing no or small amounts of protein C, some sporadic case reports on thromboembolic events involving "modern"

PCC preparations containing anticoagulants suggest that these complications cannot be prevented completely by these ingredients [37]. Since studies comparing PCC preparations containing different amounts of protein C or antithrombin are lacking, the protective effect of such ingredients cannot be quantified yet.

To our knowledge, our data represent the results of the first prospective study on thrombogenicity of modern PCCs in critically ill patients with acquired coagulation factor deficiencies. The administration of PCCs efficiently corrected the laboratory signs of the coagulopathy. On the other hand, administration of PCCs induced thrombin generation with the biochemical signs of a prethrombotic state, which could involve the risk of clinically relevant intravascular thrombin formation and ensuing thrombosis. No evidence for induction of DIC was found. In situations when a rapid correction of deficiencies in vitamin K-dependent coagulation factors is indicated and administration of fresh frozen plasma seems unreasonable due to volume overload or time loss, administration of PCCs can be an effective therapy. However, use of the lowest effective dose and use of preparations known to contain small amounts of activated coagulation factors could be particularly beneficial in the light of intravascular thrombin formation. Knowledge about dosing, indications and possible risks associated with the use of PCCs, as well as careful monitoring of the patient to detect any thromboembolic episodes, should be mandatory.

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