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PROTEIN C CONCENTRATE AS ADJUVANT TREATMENT IN NEONATES WITH SEPSIS-INDUCED COAGULOPATHY: A PILOT STUDY

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ABSTRACT—The objective of the study is to describe safety and effects of protein C concentrate (PCConc) administration in neonates with sepsis-induced coagulopathy. Eighteen neonates (12 preterm and 6 full term) aged between 1 and 28 days who have severe sepsis (n = 6) or septic shock (n = 12), with coagulopathy and acquired protein C (PC) deficiency received PCConc (i.v. bolus of 100 IU/kg, followed by 50 IU/kg every 6 h for 72 h). Platelet counts, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer, C-reactive protein (CRP), antithrombin (AT), PC, CRP, and neonatal therapeutic intervention scoring system (NTISS) were assessed before and 24, 48, and 72 h after the study entry. According to Clinical Risk Index for Babies II score (CRIB II score), the expected mortality in preterms was 10%. After 24 h of treatment, PC activity levels increased from an average of 19% to 57%, and they were within normal limits before the last PCConc bolus. During the treatment period, a shortening of PT ($P = 0.04$) and activated partial thromboplastin time ($P = 0.02$), and an increase in antithrombin levels ($P < 0.0001$) were observed, along with a reduction in CRP ($P = 0.005$) and NTISS values ($P = 0.003$). No adverse events were observed. This pilot study shows that in neonatal severe sepsis, normalization of PC levels is safe and probably effective in modulating the inflammatory response and in controlling coagulopathy. However, for the potential beneficial effects of PCConc administration on morbidity and mortality, a placebo-controlled, double-blind study is required.

KEYWORDS—Neonatal severe sepsis, coagulopathy, protein C, protein C concentrate

ABBREVIATIONS—APTT–activated partial thromboplastin time; AT–antithrombin; CRP–C-reactive protein; FFP–fresh frozen plasma; NICU–Neonatal Intensive Care Unit; NTISS–neonatal therapeutic intervention scoring system; PC–protein C; PCConc–protein C concentrate; PRBC–packed red blood cells; PT–prothrombin time; WBC–white blood cell count

INTRODUCTION

Of all the infections occurring during the first year of life, more than half pertain to the neonatal period. In neonatal intensive care units (NICUs), between 33% and 66% of newborns develop an infection, and in 50% of cases, this is associated with sepsis (1). In the United States, severe sepsis occurs in 0.3% of babies' live births (1), and the associated mortality rate (20%–40%) (2, 3) has not shown a significant reduction over the last 25 years despite the use of broad-spectrum antibiotics and support treatments (4).

Several studies indicate that the mechanisms of sepsis include excess activation of the coagulation cascade, inhibition of endogenous natural anticoagulants, and impaired fibrinolysis (5). Within the microcirculation, this leads to fibrin deposition, contributing to hypoperfusion that eventually results in tissue damage and organ dysfunction (6). On the other hand, consumption of coagulation factors and platelets promotes a bleeding tendency that may clinically manifest as petechiae, ecchymoses, and sometimes hemorrhages, all of which are associated to increased mortality.

The strong activation of coagulation during sepsis results in depletion of protein C (PC), a vitamin K–dependent natural anticoagulant, which exerts a crucial role in the modulation of coagulation, fibrinolysis, and inflammation (7, 8). Markedly reduced PC levels predict a poor clinical outcome in severe meningococcemia and infectious purpura (9, 10), and in severe sepsis due to a variety of other infectious agents (11–13). In addition, in neonatal sepsis, reduced PC activity levels are associated with increased mortality (14).

Infusion of activated recombinant human PC in adult severe sepsis is associated with an improvement in markers of coagulopathy and inflammation, and, more important, with reduced mortality (15). However, in a large trial of pediatric sepsis, the safety profile of activated PC was considered unacceptable in children younger than 60 days due to an increased incidence of intracranial bleeding (16).

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There is a clear rationale for the restoration of physiologic anticoagulant pathways in patients with sepsis and coagulopathy (17, 18), and a number of reports have suggested that in patients with meningococcemia, the infusion of PC concentrate (PCConc), aimed at normalization of PC zymogen levels, may result in markedly reduced morbidity and mortality (19, 20).

We report the results of an observational pilot study conducted on a group of newborn infants with severe sepsis of nonmeningococcal etiology, coagulopathy, and low PC levels treated with a PCConc. Our aim was to evaluate whether PCConc supplementation could safely lead to a prompt improvement in markers of coagulation, inflammation, and in overall indices of disease severity.

PATIENTS AND METHODS

In our study protocol, neonates received PCConc (Ceprotin; Baxter, Vienna, Austria) if they fulfilled the criteria of severe sepsis and clinical or laboratory signs of coagulopathy. Sepsis was judged severe when complicated by cardiovascular dysfunction, acute respiratory distress, or dysfunction of two or more organs (respiratory, renal, neurological, hematological, or hepatic) (21). Septic shock was defined according to criteria established by the 2004 International Forum on Sepsis (22). Laboratory markers of coagulopathy were prothrombin time (PT) and activated partial thromboplastin time (aPTT) ratios greater than 1.18 and 1.12, respectively, platelet counts lower than 140,000/dL, fibrinogen levels lower than 0.16 g/dL, D-dimer levels higher than 0.5 mg/L, and antithrombin (AT) and PC levels lower than expected based on gestational age and days of life (23).

The mortality risk in preterm babies was calculated by using the Clinical Risk Index for Babies II score (CRIB II score) (24) and the Score for Neonatal Acute Physiology, Perinatal Extension, version II (SNAPPE-II) (25). In addition, the neonatal therapeutic intervention scoring system (NTISS) (26) was monitored before PCConc administration and on the following days of treatment.

All neonates received conventional therapy (antibiotics, saline, vitamin K), and, if required, transfusions of packed red blood cells, platelets, and/or fresh frozen plasma (FFP), inotropic support (dobutamine, dopamine), and/or assisted mechanical ventilation. Packed red blood cells, platelet concentrates, and FFP were transfused to maintain hematocrit values above 35%, platelet counts above 100,000/dL and fibrinogen levels above 0.15 g/dL, respectively.

Within 24 h from diagnosis of severe sepsis, the PCConc was administered as an intravenous bolus of 100 IU/kg over 1 h, followed by doses of 50 IU/kg every 6 h for 72 h, with the aim to attain PC activity levels within the normal range for gestational and neonatal age (24). Thirty minutes before the administration of PCConc (baseline) and then at 24, 48, and 72 h, 2.8-mL blood samples were taken from all neonates for the measurement of PT, aPTT, fibrinogen, D-dimer, AT, PC, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, C-reactive protein (CRP), hematocrit, white blood cell (WBC), and platelet counts.

Coagulation parameters were measured in citrated plasma using an automated compact BCS analyzer (Dade Behring SpA, Milan, Italy). The activity of PC and of AT was determined by amidolytic methods (Chromogenix, Instrumentation Laboratory, Milan, Italy, and Behrichrom Antitrombin III, Dade Behring). Fibrinogen was measured by a modified Clauss method (Multifibren U). D-Dimer was measured by an immuno-enzymatic method (mini VIDAS, bioMèrieux, Rome, Italy). The remaining parameters were determined by standard procedures using automated instrumentation.

Parental informed consent for off-label use of PCConc and blood drawing for laboratory tests was obtained before enrolment in all participant NICUs.

Statistical analysis

For descriptive purposes, all continuous variables are reported in tables and graphs as mean ± SD or mean and range. The Kolmogorov-Smirnov test was used to assess normality of the distribution of continuous variables. Nonnormally distributed variables (D-dimer, CRP, WBC, creatinine, AST, ALT) were log-transformed to approximate normal distributions. Changes in variables throughout PCConc treatment were assessed by analysis of variance for repeated measures. With P values less than 0.05 at ANOVA, post hoc analyses of changes over baseline (pretreatment levels) at 24, 48, and 72 h were performed by the paired Student t test with Bonferroni correction, and differences were considered to be statistically significant at the 0.05 level.

RESULTS

During a 16-month period, 18 newborns (11 boys and 7 girls) were enrolled from 6 NICUs. Twelve preterm neonates had an average gestational age of 29.7 weeks (range, 25–35 weeks) and a birth weight of 1,283 g (range, 586–2,230 g); six full-term neonates (gestational age, 38.7 weeks; range, 38–40 weeks) had an average birth weight of 3,220 g (range, 2,800–4,100 g).

At diagnosis of severe sepsis, the 18 neonates had an age of 1 to 28 days. Three of them were individual twins from different pairs. Twelve were born by cesarean section. Two neonates had two organ failures, 12 neonates had 3 organ failures and 4 neonates had 4 organ failures. Twelve babies had septic shock. The average CRIB II score and SNAPPE-II, in preterms were 6 (range, 1–12) and 32.8 (range, 10–64), respectively, for an expected death rate of 8% to 10%. The etiology of infection was documented by blood and/or cerebrospinal fluid cultures in six cases (*Streptococcus agalactiae*, n = 2; *Escherichia coli*, n = 1; *Lysteria monocytogenes*, n = 1; *Klebsiella pneumoniae*, n = 1; *Enterobacter cloacae*, n = 1). Bleeding manifestations had occurred in six neonates; intraventricular hemorrhage in two, petechial rash in two, lung hemorrhage in one, and gastric hemorrhage in one.

The 12 neonates who suffered shock were treated with inotropic drugs, started at admission, and continued for a mean of 5 (range, 4.3–8.7) days. Because of respiratory distress and progressive hypoxemia, 16 neonates received mechanical ventilation for a mean of 8.5 (range, 4–17) days. Thirteen neonates received transfusions of FFP and 10 of packed red blood cells and/or platelet concentrates. One neonate, with bleeding manifestations, received AT concentrate because of a significant decrease in AT activity (26%). The length of stay in NICU averaged 72 (range, 28–119) days.

Before starting treatment with PCConc, PC activity was severely reduced in all neonates (19 ± 8%), and it was associated with prolongations of PT (ratio, 1.81 ± 1.04) and aPTT (ratio, 1.81 ± 0.83), increased D-dimer levels (2.69 ± 2.98 mg/L), reduced platelet counts (92,000 ± 84,000/μL), and AT levels (39 ± 13%).

The intravenous administration of PCConc started within 24 h from diagnosis of severe sepsis, and treatment was continued for 72 h. The total amount of PCConc infused averaged 1,500 IU (range, 420–2,830 IU). No neonate dropped out of the study due to clinical worsening, and all neonates completed the PCConc treatment schedule. As expected, an increased PC activity was observed during PCConc administration ($P < 0.0001$). The chosen dosage was sufficient to adjust and maintain the PC plasma activity within the normal ranges for gestational age and days of life at 24 (57 ± 20%), 48 (73 ± 28%), and 72 h (58 ± 31%; Fig. 1A), with no significant difference in PC levels in preterm and full-term babies.

During treatment, there were no significant changes in D-dimer, fibrinogen, platelet counts, hematocrit values, and WBC counts (Table 1). Conversely, a shortening of PT and aPTT (Table 1) and an increase in AT levels 48 h after the initiation of treatment were observed (Fig. 1B). AST and creatinine levels were also significantly reduced after 48 and 72 h, respectively (Table 1). Interestingly, a marker of

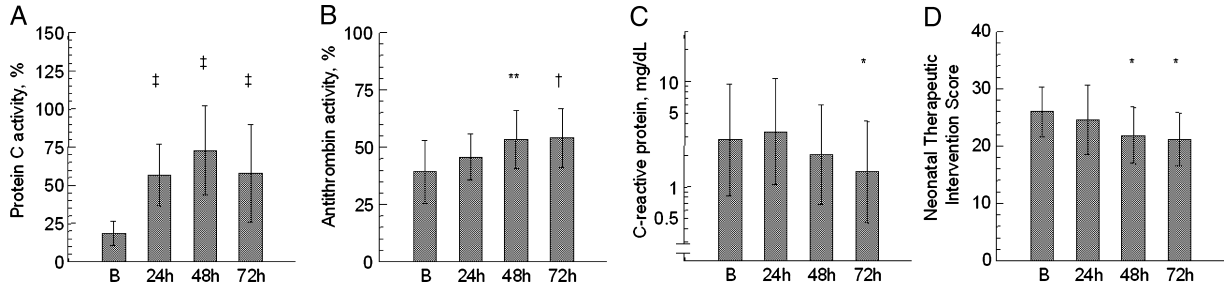

FIG. 1. **Changes in PC activity (A), AT (B), CRP (C), and NTISS (D) in 18 neonates with severe sepsis during treatment with PCConc**. The patient receiving AT concentrate was excluded from the analysis (B). CRP levels are displayed on a log-scale (C). *P* values for differences versus baseline are corrected according to Bonferroni (*<0.05; **<0.01; †0.001; ‡<0.0001).

inflammation usually considered as an indicator of sepsis severity, CRP, was significantly reduced at 72 h (Fig. 1C).

Throughout PCConc treatment, an improvement in clinical and hemodynamic conditions was observed, with a reduction of poorly perfused regions and of thermal instability, resulting in a linear decrease in requirement for assistance, as measured by the NTISS, which was already statistically significant after 48 h of treatment (Fig. 1D). Changes in pH and base deficit in the entire series of neonates, in P_{O_2}/F_{IO_2} ratio in the 16 neonates on mechanical ventilation and in MAP in the 12 neonates with shock are shown in Figure 2. Significant improvement in pH and base deficit were observed after 24 and 48 h of treatment, respectively. The average MAP increased from 26.6 at baseline to 31.3 at 24 h, and the P_{O_2}/F_{IO_2} ratio from 0.60 at baseline to 0.91 at 24 h.

No adverse reactions, bleeding, and/or thrombosis were observed during PCConc administration or thereafter, and all neonates were alive 30 days after the end of treatment. Twelve infants underwent a detailed neurofunctional evaluation at 3 months of life. It included audiometry and visual test, evoked and spontaneous motility, postural adaptability, variability of motor patterns, and neuromotor and behavioural skills. Eight had a normal developmental test, three showed minor neurologic deficiencies (impairment of motor, postural, adaptative functions) and one neonate, who had suffered intraventricular hemorrhage and convulsions, showed major neurological abnormalities.

DISCUSSION

PCConc administration is the treatment of choice in neonates with purpura fulminans who are homozygous—or compound heterozygous—for PC deficiency. In these very few patients, PC replacement prevents progression of microvascular thrombosis with rapid improvement of cutaneous lesions (27–29). Accordingly, infusion of PCConc has also been successfully attempted in conditions of acquired severe PC deficiency, like meningococcal sepsis where PC depletion is extremely severe, skin lesions are frequently observed, and PC levels are inversely related to mortality. Although low PC levels are also associated with increased mortality in neonatal sepsis of nonmeningococcal etiology (14), there is only scant, if any, information on the administration of PCConc in this clinical setting.

Uncontrolled pilot studies such as ours, far from proving clinical efficacy in terms of mortality and morbidity, may nonetheless provide information regarding the biological effects of PCConc administration. In our series of neonates with severe sepsis treated with PCConc for 72 h, we observed a rapid improvement in global coagulation tests (PT, aPTT) and a significant reduction in CRP, a marker of inflammation

TABLE 1. **Changes in laboratory variables during treatment with PCConc in 18 neonates with severe sepsis**

	Baseline	24 h	48 h	72 h	*P**
PT ratio	1.81 ± 1.04	1.42 ± 0.50	1.34 ± 0.48**	1.26 ± 0.29**	0.039
aPTT ratio	1.81 ± 0.83	1.49 ± 0.55	1.44 ± 0.58**	1.42 ± 0.52**	0.022
D-Dimer (mg/L)	2.69 ± 2.98	2.95 ± 2.46	2.50 ± 2.09	2.66 ± 2.56	0.366
Fibrinogen (g/dL)	0.28 ± 0.12	0.36 ± 0.12	0.33 ± 0.14	0.36 ± 0.14	0.308
Platelet count ($\times 10^3$/dL)	92 ± 84	111 ± 82	120 ± 90	169 ± 205	0.109
Ht (%)	42.4 ± 8.3	41.3 ± 8.0	41.2 ± 7.7	41.4 ± 8.0	0.675
WBC count ($\times 10^3$/dlL)	13.4 ± 16.5	13.7 ± 15.3	13.5 ± 10.3	15.3 ± 8.5	0.836
Creatinine (mg/dL)	6.01 ± 14.7	3.62 ± 6.76	1.87 ± 3.76	1.64 ± 2.87‡	0.042
AST (IU/L)	294 ± 485	292 ± 545	131 ± 223†	52 ± 63‡	0.0006
ALT (IU/L)	79 ± 208	73 ± 177	67 ± 123	20 ± 16	0.060

*Analysis of variance for repeated measures. For variables with *P* values less than 0.05 at ANOVA, significance of the difference over pretreatment levels at 24, 48, and 72 h is shown (*P* values with Bonferroni correction, **<0.05; †<0.01; ‡<0.001).

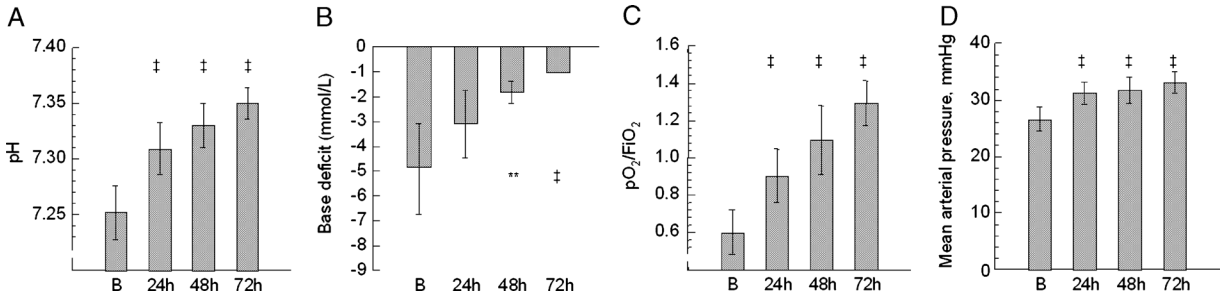

FIG. 2. **Changes in pH (A), base deficit (B), P_{O_2}/F_{IO_2} ratio (C), and MAP (D) during PCConc treatment**. Data (A and B) are from the entire series of neonates with severe sepsis; data (C and D) are from 16 neonates on mechanical ventilation and 12 neonates with shock, respectively. *P* values for differences versus baseline are corrected according to Bonferroni (*<0.01; ‡< 0.0001).

usually considered as an indicator of sepsis severity. These changes were accompanied by a decrease in AST and creatinine levels, an improvement in clinical and hemodynamic conditions, and, as a result, by a progressively reduced requirement for NTISS. These findings were not significantly different in term and preterm newborns, but given the relatively small numbers for the comparison, similarity of the response to PCConc administration in the two categories of patients cannot be taken for granted.

In the absence of a control group of untreated patients, it cannot be ruled out that such changes might have occurred irrespective of PCConc administration. However, although 10 of 18 patients had received FFP transfusions before PCConc administration, baseline PT and aPTT were abnormally prolonged, but they showed an early shortening after only 24 h of treatment. In addition, although there was no change in fibrinogen levels during treatment, AT levels increased progressively from low to near-normal for neonatal age (excluding from the analysis the one patient receiving AT concentrate supplementation). Early improvement in PT and aPTT ratios and reduced AT consumption have been also observed in a small series of adult patients who have severe sepsis after cardiac surgery and treated for 72 h with a lower cumulative dose (266 IU/kg) of PCConc (30). One third of our patients had bleeding manifestations; however, as in other reports, PCConc administration was not associated with bleeding or adverse reactions.

In line with the proven efficacy of activated PC infusion in adult patients with severe sepsis, the anticoagulant, profibrinolytic, and cytoprotective (31) effects of PC are attributed to the activated form, although the zymogen has been shown to inhibit selectin-mediated neutrophil adhesion to endothelial cells *in vitro* (32). Activated PC levels were not measured in our patients, but it is unlikely that the dosage used would have resulted in significant increase in circulating levels of the enzyme. In a placebo-controlled, dose-finding study in children with meningococcemia, De Kleijn et al. (33) observed a significant increase in circulating activated PC levels only with the administration of dosages of PC zymogen equal to or greater than 100 IU/kg every 6 h for 72 h, that is, dose regimens resulting in average plasma PC activity levels between 100% and 250%, way above those observed in our patients.

Thus, how can plasma zymogen levels such as those attained in our patients exert beneficial effects in terms of modulation of coagulation and inflammation in the management of severe sepsis? First, activation of PC in the microcirculation may be sufficient to promote local anticoagulation and fibrinolysis without necessarily giving rise to substantially increased systemic levels due to the short half-life of the enzyme within the circulation. Second, data obtained in animal models of severe sepsis indicate that cytoprotection and modulation of inflammation, rather than anticoagulation, are the activities strictly related to the survival advantage of activated PC infusion (31). In this respect, *in situ* activation of zymogen PC on endothelial cells is approximately five times more effective in protecting endothelial barrier properties than an equivalent concentration of exogenously added activated PC (34). A more recent *in vitro* study has shown that the anti-inflammatory and cytoprotective properties are solely to be attributed to the zymogen rather than to the activated form of PC (35), providing a strong mechanistic basis for the restoration of normal PC zymogen levels in severe sepsis. Similar to the decrease in CRP levels observed during PCConc administration in this study, an early drop in the plasma levels of inflammatory cytokines has been observed in the study of Crivellari et al. (30) in spite of no increase in circulating activated PC levels. Thus, it is possible that the improvement in coagulation markers observed after PCConc administration, if not strictly linked to the anticoagulant activity of endogenously activated PC, may result from the modulation of the inflammatory response elicited by the increased levels of PC zymogen.

In conclusion, in this uncontrolled pilot study, administration of PCConc in neonates with severe sepsis and coagulopathy is associated with favorable changes in coagulation and inflammatory parameters and with improvement of clinical and hemodynamic conditions without undesirable side effects. However, for translating these potentially beneficial effects in reduced morbidity and mortality, a placebo-controlled, double-blind study is required.

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