Severe sepsis in a premature neonate: protein C replacement therapy

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Treatment with activated protein C has been shown to reduce mortality in adult patients with severe sepsis but also to increase risk of bleeding. In patients with predisposition to bleeding, as in preterm infants, the inactivated form of protein C could serve as a safe therapeutic option.

We report the case of a preterm neonate who developed severe sepsis on the 28th day of life, who was successfully treated with the inactivated form of protein C for a period of 96 hours.

Key words: protein C, neonatal sepsis.

Sepsis can be defined as severe when it is associated with multiple organ dysfunction, hypoperfusion and hypotension, resulting from a generalized inflammatory and procoagulant response to infection[1]. Inflammatory and procoagulant responses are closely related, amplifying each other in a vicious cycle with potential catastrophic effect. In fact, inflammatory cytokines (tumor necrosis factor [TNF]α, interleukin [IL]-β e, IL6) activate the coagulation system and inhibit both fibrinolysis and natural anticoagulant factors[2], while thrombin stimulates inflammatory reaction, activating leukocytes and endothelium[3,4].

Activated protein C (APC) is particularly important in microcirculation, where it modulates both coagulation and inflammation[6]. In fact, APC, in collaboration with its cofactor protein S, inactivates factor Va and factor VIIIa, preventing further thrombin generation, and it promotes fibrinolysis inhibiting plasminogen-activator inhibitor 1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI). On the other hand, protein C interferes with the inflammatory response, inhibiting inflammatory cytokines production and preventing neutrophil chemotaxis[6]. APC results from activation of protein C by thrombin coupled to thrombomodulin. This process is increased by the endothelial cell protein C receptor (EPCR).

During sepsis, APC production may be down-regulated because of thrombomodulin inhibition by inflammatory cytokines and because of reduced protein C plasma concentrations[7]. Consumption of protein C occurs before consumption of other hemostatic factors such as antithrombin, fibrinogen, and factor V, therefore preceding overt clinical symptoms of severe sepsis and septic shock[8].

It has been shown in adult patients with severe sepsis and septic shock that acquired deficiency of protein C is associated with increased morbidity and mortality[9,10]. Replacement therapy with recombinant human activated protein C (rhAPC) significantly reduces mortality but may be associated with an increased risk of bleeding[11].

In the attempt to reduce hemorrhagic side effects, inactivated form of protein C has also been successfully used in a pediatric population[12].

It is well known that preterm infants are at higher risk of bleeding and physiologically have lower plasma levels of protein C[13]. Therefore, inactivated protein C could be an elective therapeutic option in septic neonates, particularly in preterm infants.

Case Report

A male preterm infant, with a birth weight of 800 g, was born at 28 weeks of gestational age by cesarean section because of maternal HELLP

(hemolysis, elevated liver function, low platelets) syndrome (PLT 69x10^9/L, AST 349 UI/L, ALT 265 UI/L, proteinuria 23.4 g/L). Apgar score was 7 at 1 min and 8 at 10 min. On admission to the neonatal intensive care unit (NICU), the baby developed respiratory distress syndrome, requiring mechanical ventilation (SIMV, Babylog, Dräger, Germany) and surfactant administration (2 doses of Curosurf: 200 mg/kg and 100 mg/kg, respectively). He was extubated and switched to nasal continuous positive airway pressure 18 hours later. Cerebral ultrasound performed on the first day of life showed bilateral grade II intraventricular hemorrhage (IVH)[14].

On the 28th day of life, because of severe and increasingly frequent apneic episodes, the baby required intubation. After one hour of mechanical ventilation on FiO_2 1.0 arterial blood gases were: pH 7.26, pO_2 56 mmHg, pCO_2 54 mmHg, HCO_3 22.1 mmol/L, and BE -3.8 mmol/L. The lactate concentration was 2.2 mmol/L. Due to suspected systemic infection (apnea, tachycardia, poor peripheral perfusion, abdominal distention), the baby was treated with vancomycin and gentamicin (afterwards the latter was replaced by meropenem), and intravenous immunoglobulin infusion was also added (Pentaglobin, 5 doses).

A Gram-negative microorganism, *Serratia marcescens*, grew in blood culture and broncho-alveolar lavage fluid. Urine and cerebrospinal fluid cultures were sterile. Laboratory data showed a high value of C-reactive protein (CRP) (33.9 mg/L), leukopenia (WBC 1840x10^9/L) with neutropenia (PMN 800x10^9/L) and anemia (Htc 26%; Hb 8.4 g/dl). Recombinant human granulocyte colony-stimulating factor (rhG-CSF) treatment and packed red blood cells were administered. Bicarbonate correction was also given because of metabolic acidosis (pH 7.23, pO_2 61 mmHg, pCO_2 41 mmHg, HCO_3 16.8 mmol/L, BE -9.8 mmol/L, lactate 6.9 mmol/L). Blood cortisol level was high (1350 ng/ml).

Echocardiography evaluation (Hewlett-Packard Image Point) showed reduced left ventricular (LV) function and dyskinetic interventricular septum on LV posterior wall.

ECHO-color Doppler sonography of splanchnic district documented low blood flow velocity in superior mesenteric artery (systolic peak 18 cm/s, end-diastolic 4.5 cm/s, mean velocity 10 cm/s, index of pulsatility [IP] 1.35) and high flow velocity in celiac axis (66 cm/s, 20 cm/s, 36 cm/s, IP 1.29, respectively). A pattern of vasoconstriction (index of resistance [IR] 0.95) was found in renal artery with normal systolic peak and low diastolic velocities (2 cm/sec). Cerebral Doppler sonography showed low systolic and diastolic velocities with normal Doppler indices (IR 0.81, IP 1.79). Because of tachycardia (heart rate [HR] 200/min), peripheral hypoperfusion and ECHO-Doppler sonographic data, a saline bolus (10 ml/kg) was given to correct the hypovolemic status.

Despite these measures, the infant's condition continued to deteriorate: oliguria appeared, acidosis persisted (pH 7.20, pO_2 60 mmHg, pCO_2 56 mmHg, HCO_3 18.5 mmol/L, BE -6.6 mmol/L, lactate 4.7 mmol/L) and hypotension appeared so that inotropic treatment was started (dopamine up to 10 µg/kg/min). The clinical course was also complicated by peripheral cyanosis, ischemic skin lesions of toes, fingers and outer ears, and liver dysfunction (AST 96 UI/L, ALT 140 UI//L, total serum bilirubin 7.7 mg/dl and direct bilirubin 3.2 mg/dl). Laboratory tests showed: PLT 31x10^9/L, APTT 64.2 s, INR 2.32, fibrinogen 227 mg/dl, D-dimers 2440 ng/L, antithrombin III 32.8%, protein C 13%, and protein S 33%. Measurement of plasma protein C activity was performed using CROMATIC™ protein C kit (Chromogenic, Milano).

Therefore, given the severity of the clinical condition and laboratory data, after obtaining informed consent from the parents, it was decided to give a bolus of 100 U/kg of inactivated protein C concentrate (Ceprotin®, Baxter Bio-Science) followed by 50 U/kg every six hours, infused during one hour[15].

Twenty-four hours after start of protein C treatment, the baby's clinical condition improved; blood pressure and diuresis normalized. Dopamine was discontinued after 48 hours and the baby was extubated after seven days. Skin lesions slowly improved during treatment with Ceprotin, totally disappearing in 10 days. Antibiotics were stopped after 12 days of treatment. Coagulation data reverted to normal values more slowly (Table I). Because of severe and persistent thrombocytopenia, six platelet transfusions were given. During the period of treatment with Ceprotin (96 hours), no bleedings were recorded and cerebral ultrasound remained unchanged (IVH grade II).

Table I. Coagulation Parameters at Various Time Points During Septic Episode

	0 h	12 h	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 12
aPTT (s)	47.4	64.2	46.9	37.7	37.4				42.7
PT (s)	21.0	27.9	21.9	22.5	16.8				14.4
Prothrombin complex (INR)	1.62	2.32	1.72	1.74	1.33				1.17
Fibrinogen (g/L)	443	227	380	291	388				427
D-dimers (ng/ml)		2440	5664	6661	5290				658
Antithrombin III (%)		32.8	15.3	31.3	32.6				
Protein C (%)		13		75	92				
Protein S (%)		33							
PLT (x10^9/L)	203	31*	15*	7*	14*	5*	17*	74*	105
WBC (x10^9/L)	1840	10900	11300	5690	3860	7020	7540	5430	12100
PMN (x10^9/L)	800	8500	9450	3810	2240	2200	1060	2800	6410

*platelet transfusion.
aPTT: Activated partial thromboplastin time. PT: Prothrombin time. PLT: Platelets. WBC: White blood cells. PMN: Polymorphonuclear leukocyte.

Discussion

Recombinant human activated protein C (drotrecogin alpha or Xigris; Eli Lilly, Indianapolis, IN) has been used recently in septic patients. In 2001, the Food and Drug Administration (FDA) approved its use in adult patients with severe sepsis or septic shock (Protein C Worldwide Evaluation in Severe Sepsis, PROWESS trial)[11]. The PROWESS study showed the beneficial effects of protein C but also warned about the increased risk of severe bleeding related to the strong anti-thrombotic effect of drotrecogin. However, the increased hemorrhagic risk seems to occur mainly in patients with predisposing factors.

For the pediatric population, a randomized clinical trial using rhAPC is still ongoing (RESOLVE study)[16]. However Barton et al.[17] reported that the risk of serious bleeding in septic children was similar to that in septic adults.

To the present, only three neonates[18-20] have been treated with rhAPC, and there was no evidence of hemorrhagic side effects. However, rhAPC therapy should be used with caution because severe sepsis in term and especially in preterm infants is commonly associated with bleeding complications[21].

Inactivated protein C (Protein C concentrate; Ceprotin) has been used recently in children with severe meningococcal sepsis by de Kleijin et al.[12]. In this study, inactivated protein C was demonstrated to be an efficacious and safe therapeutic option. In fact, the resolution of coagulation imbalances was observed and no severe bleeding episodes were reported.

We report a case of severe sepsis by *Serratia marcescens* in a very preterm infant with IVH treated with inactivated protein C. Treatment was started since the baby was considered to be in a life-threatening condition and low plasma level of protein C was documented. In our case, the low level of protein C and the high level of D-dimers appeared earlier than any other abnormalities of coagulative tests and thrombocytopenia. Indeed, hypotension also occurred later.

Coincidental to the low plasma level of protein C, hyperemia of celiac axis by ECHO-Doppler ultrasound was also documented. This splanchnic hemodynamic modification is consistent with data found in perinatal sepsis

by Kempley[22], and indicates very intense inflammatory response in the liver and spleen. Furthermore, a similar hemodynamic pattern has been documented in adult systemic inflammatory response syndrome[23].

No hemorrhagic signs were observed during and after treatment with Ceprotin; the pre-existing ventricular bleeding remained unchanged. Furthermore, the ischemic skin lesions did not evolve into necrosis and resolved within 10 days.

Although several therapeutic options (inotropes, antibiotics, mechanical ventilation, and replacement treatment of coagulopathy) contributed to the successful management of this septic neonate, inactivated protein C administration was shown to be a safe and efficacious treatment.

The encouraging results of our case treated with inactivated protein C and the three septic neonates treated with rhAPC lead us to believe that the time is ripe to evaluate efficacy and safety of this therapy in septic term and preterm newborns by randomized controlled clinical trials.

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