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CASE STUDY

Inhibition of granulocyte activation by surfactant in a 2-yr-old female with meningococcus-induced ARDS

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Inhibition of granulocyte activation by surfactant in a 2-yr-old female with meningococcus-induced ARDS. F.K. Tegtmeyer, J. Möller, P. Zabel. ©ERS Journals Ltd 2002.

ABSTRACT: Activated polymorphonuclear neutrophils (PMNs) play a crucial role in acute respiratory distress syndrome (ARDS) *via* extracellular release of reactive cell products such as elastase. Surfactant has proved valuable in restoring lung function in ARDS. The significance of its immunomodulatory properties with respect to this effect has not yet been clarified. The aim of the present study was to determine the anti-inflammatory effects of surfactant administration in an infant with ARDS.

During the acute phase of ARDS in a 2-yr-old female, levels of PMN-derived elastase complexed with α_1-protease inhibitor (E-α_1PI) were measured in both arterial and central venous blood samples obtained simultaneously. The results were correlated with oxygen demand and plasma concentrations of tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) after endotracheal administration of surfactant (Alveofact® 60 mg·kg·body weight^{-1}).

In the present case, for the first time, a higher E-α_1PI concentration was detected in arterial blood (4.51 mg·L^{-1}) than in central venous blood (2.28 mg·L^{-1}). After administration of surfactant, these concentrations and the arteriovenous difference decreased, indicating that during ARDS, most PMN degranulation takes place in the pulmonary vascular bed and is inhibited by surfactant administration. Simultaneously, TNF-α and IL-6 plasma concentrations decreased within hours and lung function was restored.

This local inhibition of polymorphonuclear neutrophil activation by exogenous surfactant may play a key role in the early improvement in lung function after surfactant administration.

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Acute respiratory distress syndrome (ARDS) as a complication of meningococcal septicaemia increases mortality by >50% [1]. Polymorphonuclear neutrophils (PMNs), as effector cells of the primary immune reaction, play a leading role in the pathogenesis of ARDS [2, 3]. When activated by endotoxins, these cells are sequestered in the pulmonary circulatory system. Extracellular release of proteases, inflammatory mediators and reactive oxygen metabolites lead to disintegration of the alveolocapillary barrier, resulting in the formation of pulmonary oedema and inactivation of pulmonary surfactant [4]. Endotracheal surfactant replacement is successfully used to treat the resulting respiratory distress [5, 6]. The underlying systemic inflammatory response is detectable *via* an intravascular increase in levels of PMN degranulation products, such as elastase [7], which, if not complexed with α_1-protease inhibitor, may cause tissue damage and degradation of serum proteins and coagulation factors [8]. Plasma concentrations of the elastase/α_1-protease inhibitor complex (E-α_1PI) [9] and proinflammatory cytokines from sources other than PMNs, such as tumour necrosis factor-α (TNF-α) [10] and interleukin-6 (IL-6) [11], were shown to correlate with the severity of disease. The authors' own *in vitro* results have led them to assume that a key effect of surfactant treatment of ARDS may be related to inhibition of PMN activation [12]. However, this has not yet been studied in patients. Therefore, in arterial and central venous blood samples, which were taken simultaneously during the course of meningococcal sepsis-induced ARDS, levels of E-α_1PI, as an indicator of PMN activation, and proinflammatory cytokines, such as TNF-α and IL-6, were determined and evaluated in relation to surfactant therapy and oxygen demand.

Case report

A female aged 2 yrs and 9 months was admitted to the paediatric intensive care unit of the Medical University of Lübeck with rapidly increasing apathy and vomiting following a brief period with feverish cold symptoms. She had not previously received any treatment. Her peripheral pulses were imperceptible and her cardiac frequency was 125 beats·min^{-1}. At this time, the child's reactions to painful stimuli were only

unspecific; her respiration was rapid and shallow. Central body temperature was raised but the cool skin at the periphery already showed multiple petechiae and ecchymoses. The Glasgow Meningococcal Septicaemia Prognostic Score, with 15 out of a possible 15 points, indicated that a fatal outcome was to be expected [13].

Laboratory analysis on admission

The following values were obtained from arterial blood analysis on admission: pH 7.15; arterial carbon dioxide tension 5.68 kPa; arterial oxygen tension (P_{a,O_2}) 2.88 kPa; base excess -14 mM; lactate 3.4 mM; glucose 1.78 mM; granulocytes 12,900 cells·μL^{-1}, of which 17% were immature precursors; thrombocytes 165,000 cells·μL^{-1}; fibrinogen 138 mg·dL^{-1}; fibrinogen degradation products >8 mg·L^{-1}; international normalized prothrombin ratio 3.1; partial thromboplastin time 77.5 s; aspartate aminotransferase 13 U·L^{-1}; alanine aminotransferase 7 U·L^{-1}; γ-glutamyltransferase 8 U·L^{-1}; lactate dehydrogenase 266 U·L^{-1}; sodium 145 mM; potassium 4.55 mM; calcium 1.36 mM; chloride 100 mM; TNF-α 1.56 ng·mL^{-1} (enzyme-linked immunosorbent assay); IL-6 9.59 μg·mL^{-1} (bioassay); C-reactive protein (CRP) 18 mg·L^{-1}; and E-α_1PI 1.85 mg·L^{-1}. The following values were obtained in cerebrospinal fluid: protein 0.82 g·L^{-1}; glucose 96 mg·dL^{-1}; lactate 2.5 mM; and leukocytes 1,365 cells·μL^{-1}, of which 63% were PMNs. Both blood and cerebrospinal fluid cultures resulted in growth of *Neisseria meningitidis* group B.

Course

Immediately after admission, oxygen was given, a central venous and an arterial catheter were inserted and the child was intubated and ventilated. After initial blood samples had been taken, dexamethasone was given (0.6 mg·kg·body weight^{-1}·day^{-1} in four separate doses) and antibiotic therapy was started with cefotaxime (200 mg·kg·body weight^{-1}·day^{-1} in four individual doses) and penicillin (500 mg·kg·body weight^{-1}·day^{-1} in six individual doses). Circulation was restored by infusions of sodium chloride 0.9%, catecholamines and fresh frozen plasma. Ventilatory requirements increased and progressive respiratory distress developed rapidly (fig. 1). Eight hours after admission (peak inspiratory pressure 40 cmH_2O, positive end-expiratory pressure 8 cmH_2O, mean airway pressure (MAP) 18 cmH_2O, inspiratory oxygen fraction (F_{i,O_2}) 1.0, $P_{a,O_2}/F_{i,O_2}$ 11.2 kPa, oxygenation index (OI; 100 $F_{i,O_2}MAP/P_{a,O_2}$) 21), surfactant was given endotracheally (Alveofact®; Boehringer Ingelheim Corp., Ingelheim, Germany) at a total dose of 60 mg·kg·body weight^{-1}. Two hours after surfactant administration, there was clear improvement in lung function ($P_{a,O_2}/F_{i,O_2}$ 16.1 kPa, OI 16) with further subsequent recovery after 12 h ($P_{a,O_2}/F_{i,O_2}$ 23.9 kPa, OI 8). The child was extubated on the ninth day of treatment.

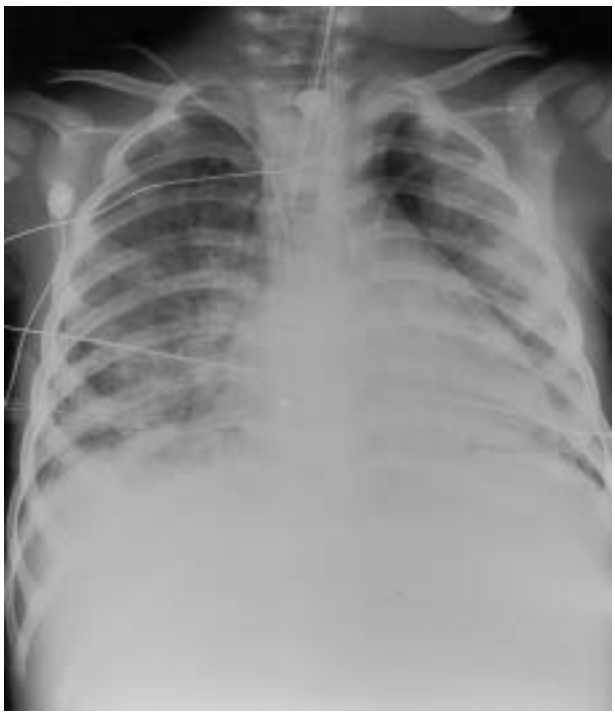

Fig. 1.–Chest radiography of a 2-yr-old female with sepsis-induced acute respiratory distress syndrome before surfactant administration.

With regard to the changes in chemical concentrations, although, initially, CRP concentration was only moderately raised, the E-α_1PI concentration was already pathologically elevated at 1.85 mg·L^{-1} (reference range 80–230 μg·L^{-1}), and both TNF-α (1.56 ng·mL^{-1}) and IL-6 (9.59 μg·mL^{-1}) had already reached maximum plasma concentrations (fig. 2). Five hours after admission, the venous E-α_1PI concentration decreased from its maximum of 4.18 mg·L^{-1} to 2.29 mg·L^{-1} 3 h later, while the arterial E-α_1PI concentration was still increasing from 4.27 mg·L^{-1} to a maximum level of 4.51 mg·L^{-1}. At this time, administration of surfactant led to an immediate improvement in lung function associated with a reduction in arteriovenous E-α_1PI difference, to approximately a quarter of the maximum value within 2 h and its further decrease during the course of treatment (table 1). These changes were also accompanied by a clear reduction in the arterial E-α_1PI, IL-6 and TNF-α plasma concentrations. The increase in the number of circulating PMNs from an initial 12,900 cells·μL^{-1} to 17,500 cells·μL^{-1} at the time of surfactant therapy remained stable throughout treatment. CRP concentration increased continuously to 348 mg·L^{-1} within 48 h. In spite of effective restoration of circulation and optimization of peripheral perfusion by local administration of nitroglycerine and anticoagulation with heparin (100 U·kg^{-1}·24 h^{-1}), marked skin necroses developed, which healed leaving scars. The child survived in spite of the unfavourable prognosis. Recovery, however, was poor and comprised severe hypoxic/ischaemic encephalopathy, marked spasticity and absence of an interactive response.

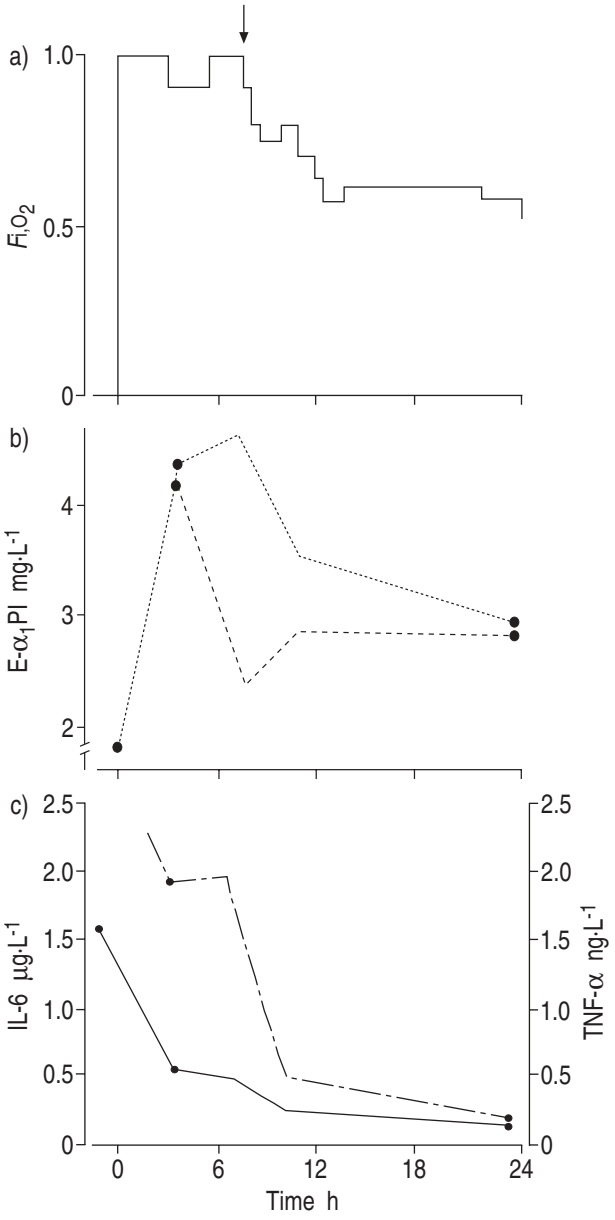

Fig. 2. – Time course of: a) inspiratory oxygen fraction (F_{i,O_2}); b) arterial (·····) and central venous (– – –) elastase/α_1-protease inhibitor complex (E-α_1PI) plasma concentration; and c) arterial tumour necrosis factor-α (TNF-α) (– - – -) and interleukin-6 (IL-6) (——) plasma concentration after endotracheal surfactant administration (vertical arrow) in a 2-yr-old female with acute respiratory distress syndrome.

Discussion

Currently, ARDS is still a mostly fatal complication of meningococcal septic shock [1]. The correlation known to exist between the severity of the clinical symptoms, the plasma concentration of proinflammatory cytokines and mortality led to the expectation of a fatal outcome in the present case [1, 11]. As in previously reported cases, the patient already showed the maximum increase in TNF-α and IL-6 concentrations on admission [11]. In addition, the PMN activation could be recorded on the basis of the rapidly increasing E-α_1PI concentrations and correlated with the respiratory distress both clinically and radiologically.

As reported previously, cytological and biochemical analyses of bronchoalveolar lavage fluid provided evidence of pulmonary sequestration of PMNs and high concentrations of their cell products [1]. The pulmonary site of PMN activation in ARDS has also been reported by ZIMMERMANN *et al.* [14], who demonstrated that the *in vitro* activation of arterial PMNs was greater than that of central venous PMNs. In the present case, too, the lung could be identified as the site of maximum PMN activation *via* determination of arteriovenous E-α_1PI difference as a result of *in vivo* pulmonary PMN degranulation. To the present authors' knowledge, there have been no reports describing such an arteriovenous difference in concentration of E-α_1PI as an indicator of pulmonary PMN degranulation to date. Although the endotracheal administration of natural surfactant proved to be an effective component of the treatment of ARDS [5, 6], because of its biophysical properties [15], the part played by the numerous biological anti-inflammatory effects of this substance with regard to the acute effect still has to be elucidated. Suppression of lymphocyte and monocyte function [16], as well as inhibition of cytokine-induced PMN activation [6] by surfactant, has not yet been examined in patient studies.

In the case described here, for the first time, changes in lung function, pulmonary release of elastase and cytokine concentration in relation to surfactant treatment can be documented on the basis of a synoptic record of the course of the disease.

The rapid decrease in arterial elastase/α_1-protease inhibitor complex concentration and the elimination of its arteriovenous difference in association with

Table 1. – Time course of parameters measured relative to therapy in a 2-yr-old female with acute respiratory distress syndrome

Time after admission n	$P_{a,O_2}/F_{i,O_2}$	Arterial E-α_1PI µg·L^{-1}	Venous E-α_1PI µg·L^{-1}	TNF-α ng·L^{-1}	IL-6 µg·L^{-1}	CRP mg·L^{-1}
0.5	75	1.85		1.56	9.59	18
5	133	4.25	4.18	0.525	1.91	
8*	84	4.51	2.29	0.465	1.96	121
10	121	3.43	2.82	0.246	0.487	180
28	276	2.68	2.17	0.043	0.044	274

P_{a,O_2}: arterial oxygen tension; F_{i,O_2}: inspiratory oxygen fraction; E-α_1PI: elastase/α_1-protease inhibitor complex; TNF-α: tumour necrosis factor-α; IL-6: interleukin-6; CRP: C-reactive protein. *: before surfactant administration.

the reduction in circulating cytokine concentration following surfactant administration, strongly suggest inhibition of polymorphonuclear neutrophil activation of the lung. These changes and their correspondence with lung function improvement may indicate that the anti-inflammatory properties of surfactant are involved in the acute effect of this substance. Even if interaction with cells of the alveolar space, such as macrophages, granulocytes and pneumocytes, is the primary effect because of the topical administration of surfactant, with regard to impairment of the alveolocapillary barrier in acute respiratory distress syndrome, the possibility of an extra-alveolar effect of surfactant components on interstitial and intravascular cells cannot be ruled out. The administration of surfactant appears to affect not only activation of polymorphonuclear neutrophils but also the response of other inflammatory cells contributing to the increase in tumour necrosis factor-α and interleukin-6 concentrations. Since tumour necrosis factor-α and interleukin-6 are among the target substances of the cytokine-blocking strategies currently being developed [17], surfactant administration may offer further options when used in conjunction with such therapy. Further studies are needed to address questions regarding whether the effects observed in the present case apply similarly to other surfactant preparations, which components of surfactant are involved in these effects, especially whether a local reduction in surfactant protein A concentration might play a permissive role in these effects, and whether the desired effects could be optimized by modification of the dose and dosing regimen.

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