Plasma concentration of elastase- α_1 -proteinase inhibitor complex in surfactant-treated preterm neonates with respiratory distress syndrome

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Plasma concentration of elastase- α_1 -proteinase inhibitor complex in surfactant-treated preterm neonates with respiratory distress syndrome. F.K. Tegtmeier, J. Möller, A. Richter, B. Wilken, Th. Fischer. ©ERS Journals Ltd 1994.

ABSTRACT: Although exogenous surfactant replacement improves respiratory distress syndrome (RDS) of immature neonates, it may not prevent subsequent lung damage and development of bronchopulmonary dysplasia associated with polymorphonuclear neutrophil (PMN)-activation. We therefore wanted to assess whether surfactant administration would be associated with activation of circulating PMNs.

Since elastase- α_1 -proteinase inhibitor (E- α_1 -PI) has proved to be a sensitive indicator of intravascular PMN activation, we studied E- α_1 -PI plasma concentration in preterm neonates during the treatment of RDS with a bovine surfactant preparation (group I: n=23). Results were compared with those from a retrospective control group treated by ventilation alone (group II: n=13), and with a reference group of 92 newborns (group III).

Following surfactant administration, median E- α_1 -PI concentration increased significantly (day 1 80.5 vs Day 2 234 $\mu\text{g}\cdot\text{L}^{-1}$), and exceeded the upper limit of the reference range of 274 $\mu\text{g}\cdot\text{L}^{-1}$ in seven patients, with a maximal value of 1,881 $\mu\text{g}\cdot\text{L}^{-1}$ after multiple surfactant administrations. In contrast, 12 infants from Group II showed no increase in median E- α_1 -PI levels (Day 1 107 vs Day 2 107 $\mu\text{g}\cdot\text{L}^{-1}$), and remained within the reference range (Day 1 125 $\mu\text{g}\cdot\text{L}^{-1}$; Day 2 107 $\mu\text{g}\cdot\text{L}^{-1}$) of the 92 newborns without respiratory impairment, infection, birth-trauma or asphyxia.

From these results, it is concluded that surfactant may trigger a transient, mainly local, inflammatory response, reflected by increased levels of E- α_1 -PI, and may exert a dose-related pathogenic influence on the course and prognosis of RDS. Under these conditions, the validity of E- α_1 -PI for the diagnosis of early-onset septicaemia may be limited.

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Endotracheal administration of bovine, porcine or human surfactant preparations has proved to be an effective therapeutic approach for the idiopathic respiratory distress syndrome (RDS) in immature neonates [1]. Until now, major attention has been focused on improvement of respiratory function as the most important factor for survival. However, immunological side-effects induced by surfactant constituents have received little attention. Phagocytosis of surfactant by pulmonary macrophages [2, 3], antibody formation against surfactant components [4], enhancement of pulmonary leucostasis following surfactant replacement [5], and contamination of natural surfactant preparations with platelet-activating factor (PAF) [6] have been observed, and raise the question of the induction of an inflammatory response by such treatment.

Since phagocytosis may be associated with cytokine release, uptake of surfactant by pulmonary macrophages may lead to cytokine-mediated polymorphonuclear neutrophil (PMN) activation, followed by a potentially harm-

ful release of enzymes and oxygen metabolites from these cells [7–9]. This process may be enhanced by PAF, which has been found to induce the expression of proadhesive molecules for PMN on endothelial cells [10].

Elastase, a neutral protease stored in the azurophilic granules of PMNs, is a major release product. In its active state, this enzyme exerts tissue destructive properties and immunomodulatory effects [8, 9, 11]. After complex formation with its major inactivator, α_1 -proteinase inhibitor, to form elastase- α_1 -proteinase inhibitor (E- α_1 -PI) it has proven to be a most sensitive indicator of PMN activation during septicaemia in neonates, and of tissue damage following surgical trauma in adults [12, 13].

We therefore studied the plasma concentration of E- α_1 -PI during the treatment of RDS with a bovine surfactant preparation, in order to assess a potential surfactant-related PMN activation. We speculated that this may contribute to the development of subsequent chronic lung disease.

Patients

Thirty six neonates, admitted to the intensive care unit of the Department of Pediatrics and Neonatology of the Medical University of Luebeck from 1987 to 1991, constituted our study group. Of these, 23 were preterm patients showing RDS (group I: gestational age 30.2 ± 2.3 weeks (mean $\pm$ SD)), treated by endotracheal administration of a bovine surfactant preparation (Alveofact®, Thomae Co., Biberach/Riss, Germany). Group I was compared with a retrospective control group of preterm neonates with the same diagnosis (group II: $n=13$; gestational age 31.2 ± 2.5 weeks (mean $\pm$ SD)), treated only by artificial ventilation during the period prior to 1989, when surfactant treatment became available to us. Diagnostic approaches, regimens of ventilation, antibiotic treatment, and gestational age-related mortality were the same for both groups during the study periods. Patient selection was based on diagnostic criteria of RDS, including the typical clinical findings and radiological changes according to COUCHARD *et al.* [14].

Methods

Surfactant was administered according to the criteria used by HORBAR *et al.* [15]. Treatment was started as a rescue regimen within 8 h postpartum, by endotracheal administration of $50 \text{ mg} \cdot \text{kg}^{-1}$ surfactant preparation. Four patients were treated repeatedly, reaching cumulative doses of $200 \text{ mg} \cdot \text{kg}^{-1}$ in one, $150 \text{ mg} \cdot \text{kg}^{-1}$ in another, and $100 \text{ mg} \cdot \text{kg}^{-1}$ in two other patients. There were no significant differences between Group I and Group II with respect to gestational age, birth weight, severity of RDS defined by X-ray changes, and fraction of inspired oxygen (FIO_2) at 2 h after birth (table 1). One surfactant-treated patient, a very immature baby, (gestational age of 24 weeks) exhibited severe pulmonary emphysema, and died from cardiopulmonary insufficiency on the second day of life.

Reference values of E- α_1 -PI had been obtained from neonates (group III gestational age 36.6 ± 2.6 weeks (mean $\pm$ SD)) without symptoms of respiratory impairment and infection. These subjects were not matched for ges-

tational age, since the number of comparable immature babies without RDS was too small. The validity of such controls has not been proven, and no correlation between E- α_1 -PI-levels and maturity has been found so far. E- α_1 -PI measurements are standard routine laboratory analyses performed in all neonatal intensive care unit (NICU) patients in our department.

Exclusion criteria

Patients in whom granulocyte activation had been expected due to infection [12], traumatic or hypoxic-ischaemic tissue destruction [13–16] were excluded from the study. Infection was suspected in cases of pathological elevation of the ratio of immature to total neutrophil granulocytes (I/T-ratio >0.2) and C-reactive protein (CRP $>15 \text{ mg} \cdot \text{l}^{-1}$) on the first day of life, and positive bacterial cultures from blood, tracheal aspirates and pharyngeal swabs. Asphyxia was based on a 5 min Apgar score of <7 .

Laboratory methods

Analyses were performed from ethylenediaminetetraacetic acid (EDTA)-blood, obtained by venipuncture or from arterial lines. Measurements of E- α_1 -PI were carried out by an immunoluminometric assay, as described previously [17]. Total granulocyte count (PMN count) and the I/T-ratio were calculated from white blood cell counts, analysed automatically and corrected for nucleated red cells, and from Pappenheim-stained blood smears. CRP was measured by kinetic turbidimetry (Turbitimer®, Behring, Marburg a.d.L., Germany).

Statistics

E- α_1 -PI levels and PMN counts of both groups were compared by the Mann Whitney U-test. Calculation of differences within the groups were performed by the Wilcoxon test.

Table 1. – Characteristics of preterm neonates treated with surfactant (Group I) and without surfactant (Group II)

	Gestational age weeks	Birth weight g	Apgar score	X-ray findings*	FIO_2^{**}
Group I ($n=23$)	30.2 ± 2.3	1448 ± 472	8.3 ± 1.0	10/23	0.72 ± 0.17
Group II ($n=13$)	31.2 ± 2.5	1544 ± 500	8.4 ± 1.0	5/13	0.65 ± 0.30
	NS	NS	NS	NS	NS

Data are presented as mean $\pm$ SD. *: number of babies showing chest X-ray changes grade 3+4 (Couchard). **: fraction of inspired oxygen at 2 h after birth. NS: nonsignificant.

Results

Following surfactant treatment, median E- α_1 -PI levels increased significantly on the second day of life (Day 1 80.5 vs Day 2 234.0 $\mu\text{g}\cdot\text{l}^{-1}$, $p<0.001$). Prior to surfactant administration, all patients had E- α_1 -PI values within the reference range of up to 240 $\mu\text{g}\cdot\text{l}^{-1}$ (confidence level of 95%) on Day 1 of life (according to data reported by SPEER *et al.* [12]). In seven surfactant-treated patients, E- α_1 -PI levels exceeded the upper limit of the reference range (274 $\mu\text{g}\cdot\text{l}^{-1}$ defined by group III) on the second day of life, (fig. 1). This was comparable with those found in babies with severe bacterial infection. Five of these neonates exhibited values above 500 $\mu\text{g}\cdot\text{l}^{-1}$. The maximal E- α_1 -PI concentration of 1,881 $\mu\text{g}\cdot\text{l}^{-1}$ was observed in one out of four patients treated repeatedly with a total cumulative dose of 150 $\text{mg}\cdot\text{kg}^{-1}$ of surfactant. A relationship between the magnitude of the rise of E- α_1 -PI and the total dose of surfactant could not be confirmed because of the small number of patients treated with doses of more than 50 $\text{mg}\cdot\text{kg}^{-1}$. The only patient showing no increase after surfactant administration died on the second day of life (fig. 1). This very immature infant of 24 weeks gestational age exhibited severe neutropenia, decreasing over the first 2 days of life (day 1: 0.94×10^9 cells $\cdot\text{ml}^{-1}$; day 2: 0.13×10^9 cells $\cdot\text{ml}^{-1}$).

In contrast, E- α_1 -PI-plasma concentration in patients of Group II remained unchanged (107 vs 107 $\mu\text{g}\cdot\text{l}^{-1}$). Only one patient from this group showed an E- α_1 -PI elevation of 287 $\mu\text{g}\cdot\text{l}^{-1}$, exceeding the upper limit of the reference range on the second day of life. On the third day of life, E- α_1 -PI decreased in 16 patients treated with surfactant, while levels of five patients remained elevated. Starting from subnormal concentrations of E- α_1 -PI, the two groups of patients showed a significant difference in this analyte on the second day of life. There were no data for E- α_1 -PI levels of Group II patients for the third day of life, since these patients had not been monitored routinely for this analyte beyond the second day of life.

Fig. 1. — Plasma concentration of elastase- α_1 -proteinase inhibitor (E- α_1 -PI) complex in 23 surfactant treated (Group I) and 13 untreated (Group II) preterm neonates with idiopathic respiratory distress syndrome, during the first 3 days of life. $\square$: reference range of 92 newborns (including the values between the 2.5th and 97.5th percentiles).

On the first day of life, median PMN counts were found to be significantly lower in both groups of patients compared with those of the control group (table 2). On day 2, there was a significant simultaneous increase of PMN counts, reaching the reference range, irrespective of the treatment. Increased I/T-ratios and CRP levels were found in three patients on the day following surfactant administration (table 3), but in none of the untreated neonates. As shown in the table, these laboratory changes were short-lived and returned to normal as early as one day later.

Table 2. — Changes in E- α_1 -PI and PMN levels during the first 2 days of life in neonates with RDS

Day of life	Group	Pts n	E- α_1 -PI $\mu\text{g}\cdot\text{l}^{-1}$	PMN count $10^9\cdot\text{l}^{-1}$
Day 1	I	23	80.5 88.8 $\pm$ 47.9	2.05 3.48 $\pm$ 2.74
	II	13	107.0 95.7 $\pm$ 47.8	3.24 3.62 $\pm$ 2.32
	III	92	125 133 $\pm$ 66	7.4 8.0 $\pm$ 2.6*
Day 2	I	23	234.0 420.2 $\pm$ 489.5*	5.6 6.18 $\pm$ 3.85
	II	13	107.0 121.8 $\pm$ 66.5	5.93 5.97 $\pm$ 3.04
	III	92	107.0 125 $\pm$ 71	5.6 6.7 $\pm$ 3.1

Asterisks indicate significant differences from the other groups on the same day (* <0.05). Group I: newborns treated with surfactant; Group II: patients treated by ventilation alone; Group III: control patients. RDS: respiratory distress syndrome; E- α_1 -PI: elastase- α_1 -proteinase inhibitor; PMN: polymorphonuclear neutrophil; Pts: patients.

Table 3. – Time course of laboratory changes in haematological variables in 3 neonates with elevated CRP levels and/or increased I/T-ratios following surfactant treatment

Pts No.	Day of life	E- α_1 -PI $\mu\text{g}\cdot\text{l}^{-1}$	CRP $\text{mg}\cdot\text{l}^{-1}$	PMN count $10^9\cdot\text{l}^{-1}$	I/T-ratio
1	1	86	<5.00	4.74	0.12
	2	182	11.20	8.85	0.25
	3	180	6.00	11.70	0.11
2	1	141	<5.00	5.35	0.05
	2	162	25.10	10.60	0.06
	3	488	12.50	17.00	0.14
3	1	36	<5.00	2.05	0.00
	2	783	24.30	2.60	0.00
	3	306	18.30	6.36	0.01

Cumulative dose in Pts 1 and 3 $50\text{ mg}\cdot\text{kg}^{-1}$ each, in Pt 2 $100\text{ mg}\cdot\text{kg}^{-1}$. CRP: C-reactive protein; I/T-ratio: ratio of immaturity to total neutrophil granulocytes. For further abbreviations see legend to table 2.

Discussion

Surfactant deficiency in RDS of immature newborns is not primarily due to an inflammatory process [18]. Changes in laboratory parameters associated with RDS indicating inflammation, such as elevated CRP levels, have not yet been observed so far [19]. The theoretical concern, however, that surfactant might act as a pro-inflammatory agent on lung tissue is substantiated by our data, indicating a significant elevation of E- α_1 -PI plasma concentrations following surfactant administration, in contrast to patients treated by ventilation alone.

The observed increase of E- α_1 -PI is considered to be due to surfactant treatment, since patients under conditions associated with PMN activation other than surfactant administration, such as infection and tissue damage by trauma and hypoxia, had been excluded from the study. The decrease of E- α_1 -PI concentration in only one surfactant-treated patient was considered to be due to severe neutropenia following neutrophil storage pool exhaustion, since neutrophils are the exclusive source of plasma elastase.

Whether E- α_1 -PI increase is caused by a direct interaction of surfactant components or contaminants (such as PAF) with granulocytes, or is mediated by complement fragments, cytokines or mediators released from pulmonary macrophages or endothelial cells, remains to be answered. A possible mechanism may be phagocytosis-mediated activation of pulmonary macrophages, which were shown to ingest surfactant components, such as phospholipids and xenogenic proteins [2]. Subsequent phagocytosis-associated cytokine release may be followed by PMN activation and extracellular release of elastase. Additionally, PAF present in natural surfactant preparations [6] may trigger PMN activation and enzyme release by induction of leucocyte-endothelial cell interaction [10].

However, data reported by others show controversial results. Surfactant-associated suppression of cytokine release from isolated monocytes has been found by SPEER *et al.* [20]. These results, however, may not reflect the physiological situation, since removal of monocytes from their natural milieu has been shown to reduce their subsequent

immune response function [21]. A suggestion by others, that synthetic surfactant may not act as a pro-inflammatory agent, was based on findings in tracheal aspirates [22]. Free elastolytic activity determined in that study would not be detected as long as the inhibitor concentration in the sample is high enough to inactivate this protease by complex formation. Consequently, depending on the aim of that study, the significance of different methodological approaches to elastase determination, such as measurement of elastase activity instead of E- α_1 -PI-concentration, has to be considered.

Reduced numbers of circulating PMNs observed on the first day of life in our patients with RDS, may reflect pulmonary sequestration of these cells, as observed, in animals [7, 23]. PMN increase on the second day of life reach the median reference value in both of our RDS-groups, suggesting resolution of the pulmonary leucostasis or enhanced cell release from the bone marrow.

In contrast to changes of I/T-ratio and CRP level, the release of elastase is not dependent on the cytokine-mediated response of primary target organs, such as liver and bone marrow. Therefore, changes of the E- α_1 -PI plasma level may react more sensitively than do I/T-ratio and CRP level, as demonstrated previously in septic neonates [24]. We therefore suggest E- α_1 -PI increase to be a most sensitive parameter, reflecting intravascular activation of PMNs and a proinflammatory process following surfactant administration.

Since the extent of inflammation is dependent on quality and quantity of causative agents, a relationship between the maximum dose of surfactant administered and laboratory changes, such as E- α_1 -PI-levels, I/T-ratio or CRP levels, may be expected. In 3 of the 4 patients who received more than $50\text{ mg}\cdot\text{kg}^{-1}$ of surfactant, E- α_1 -PI concentrations exceeded the upper limit of the reference range, reading the maximum value of $1,881\text{ }\mu\text{g}\cdot\text{l}^{-1}$ after a dose of $150\text{ mg}\cdot\text{kg}^{-1}$. A dose-effect relationship may be postulated, but has to be confirmed by further studies, as well as the influence of different treatment regimens, different surfactant preparations (especially with regard to PAF-contamination) and patient-related factors,

such as clearance function of the monocyte-phagocyte system on PMN activation and E- α_1 -PI levels.

From these preliminary studies, we suggest that E- α_1 -PI elevation may reflect a transient proinflammatory response related to surfactant treatment. Thus, PMN activation may gain pathogen relevance in the course of severe RDS in surfactant-treated patients, especially in those receiving multiple high doses of surfactant. In conclusion, these results highlight an important side-effect of surfactant treatment, including the limitation of the validity of E- α_1 -PI for the diagnosis of neonatal septicaemia in surfactant-treated infants with RDS.

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