Activation of the Plasma Kallikrein-Kinin System in Respiratory Distress Syndrome

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ABSTRACT. Components of the plasma kallikrein-kinin and fibrinolytic systems together with antithrombin III were measured the first days postpartum in 13 premature babies with severe respiratory distress syndrome (RDS). Seven of the patients received a single dose of porcine surfactant (Curosurf) as rescue treatment. Nine premature babies without lung disease or any other complicating disease served as controls. There were no differences in prekallikrein values between surfactant treated and non-treated RDS babies during the first 4 d postpartum. The controls had, however, significantly higher prekallikrein values than the RDS babies already at the first day of age (mean $\pm$ SD $32 \pm 8\%$ in controls *versus* 22 ± 6 and $21.5 \pm 5\%$ in the treated and nontreated RDS groups, respectively). Plasma kallikrein activities did not differ between RDS and control patients. Plasma kallikrein inhibition values, which increased steadily in all groups, were lower in the RDS babies treated with surfactant than in controls at d 2 and 4. The degree of degradation of plasma high molecular weight kininogen was measured in RDS patients treated with surfactant and was significantly higher when compared with controls at d 1, demonstrating an increased proteolysis of kininogen to kinin early in RDS. There were no differences in plasminogen and plasmin values between RDS and control babies. This study shows that the plasma kallikrein-kinin system is activated in RDS. This system as well as the fibrinolytic system does not seem to be influenced by rescue instillation of a single dose of porcine surfactant into the lungs of premature babies with RDS. (*Pediatr Res* 32: 431–435, 1992)

Abbreviations

FiO₂, fraction of oxygen in inspired air
FXII, Hageman factor
HK, high molecular weight kininogen
RDS, respiratory distress syndrome
PaO₂, oxygen tension in arterial blood

proteins that interact in a complicated way. When activated, the contact factors plasma prekallikrein, FXII, and factor XI are converted to serine proteases that are capable of activating the complement, fibrinolytic, coagulation, and kallikrein-kinin systems (7–9). Inhibitors regulate and control the activation of the cascades. C1-inhibitor is the most important inhibitor of the contact system (10). It exerts its regulatory role by inhibiting activated FXII, FXII fragment, and plasma kallikrein (10). In addition, α_2 -macroglobulin and α_1 -protease inhibitor inhibit plasma kallikrein (11) and factor XIa (12), respectively. Contact activation initiates other proteolytic plasma cascades. Plasma prekallikrein, activated FXII, and factor XIa may convert the proenzyme plasminogen to plasmin (13, 14).

When the plasma kallikrein-kinin system is activated, HK is cleaved proteolytically and three characteristic bands are identified by electrophoresis followed by immunoblotting. The relative amount of each of these bands gives an indication of the degree of plasma kallikrein-kinin activation. Band I with molecular mass of 149 000 D represents the native form of HK; band II (molecular mass 104 000 D) and band III (molecular mass 99 000 D) are considered to represent degradation products of kininogen. The relative amounts of these two compounds are therefore of special interest when considering the rate of proteolytic cleavage of kininogen (15).

The plasma kallikrein-kinin system may play a fundamental role in the development of shock-like conditions because its activation results in the formation of the very potent vasodilator bradykinin, which increases capillary permeability as well (7, 9, 16, 17). We have previously shown that preterm babies with RDS have low plasma prekallikrein and kallikrein inhibition levels, indicating that the plasma kallikrein-kinin system might be activated in this condition (6).

The purpose of the present study was to investigate more specifically whether or not the plasma kallikrein-kinin system is activated in RDS. In addition to components of the plasma kallikrein-kinin system including HK and its degradation products, we have also determined components of the fibrinolytic system and antithrombin III in preterm babies with and without RDS. Additionally, we have studied whether rescue treatment with a single dose of porcine surfactant influences the activation of these systems.

MATERIALS AND METHODS

Patients. The study population comprised 22 preterm babies divided into three groups. Gestational age ranged between 25 and 33 wk and birth weight between 795 and 1 785 g. Group 1 ($n = 7$) and group 2 ($n = 6$) babies had RDS diagnosed according to well-established clinical and radiographic criteria (18). Only babies with severe RDS defined as oxygen requirement ≥ 0.60 and dependence on artificial ventilation were included. Group 1

Activation of the proteolytic cascade systems of plasma is associated with trauma, septicemia, adult RDS, and neonatal RDS (1–6). The contact system of plasma comprises a set of

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babies received one dose of natural surfactant as rescue treatment. Mean gestational age in this group of patients was 28.5 (SD 0.8) wk and birth weight was 1 241 (SD 134) g. Group 2 babies were not treated with surfactant. They had a mean gestational age of 28.8 (SD 3.2) wk and birth weight of 1 197 (SD 349) g.

The babies with RDS fulfilled the criteria for entering the first Collaborative European Multicenter Trial of replacement therapy with porcine surfactant (Curosurf) (19). The protocol and results from this study have previously been published in detail (19). In that study, RDS babies were randomized either to receive or not to receive a single dose of surfactant.

Group 3 ($n = 9$) consisted of controls without RDS or any other significant respiratory problems. To be enrolled as controls, the babies did not have an oxygen requirement ≥ 0.30 for more than 1 h after birth and required artificial ventilation only for stabilization during the first 2 d after birth. They had a mean gestational age of 28.9 (SD 1.8) wk and birth weight of 1 138 (SD 207) g. The mothers of three of them received dexamethasone between 1 and 7 d before giving birth. Eight of nine control babies were delivered by cesarean section. Their mean 1-min Apgar score was 6.7 (range 2–9), and the 5-min score was 8.4 (range 6–10). Four of these babies required intermittent positive ventilation of duration ranging between 1 and 36 h postpartum.

There were no significant differences between the three groups with regard to birth weight and gestational age. All babies were larger than the 2.5 percentile for weight.

Procedure. An arterial or venous umbilical catheter was inserted for clinical monitoring; the routine in our unit is to remove such indwelling catheters within 96 h if the babies do not require artificial ventilation.

All the RDS babies had indwelling arterial umbilical catheters, which were removed at latest approximately 24 h after extubation. Blood for analysis was withdrawn as soon as possible after stabilization after birth. The first blood sample (d 1) was withdrawn before the age of 12 h at a mean age of 7.5 (SD 3.0) h and then at 24-h intervals as long as an umbilical catheter remained in position. For babies receiving surfactant, the first blood sample was withdrawn 5 min before surfactant was given. All three groups were treated identically with regard to timing of blood sampling.

Catheters in control babies were removed between 72 and 96 h of age and data are therefore given only until d 4 after birth.

From the catheter, 0.9 mL of blood was withdrawn, avoiding contact with any heparin, and put into soft plastic tubes with 0.1 mL 3.2% citrate. After centrifugation, plasma was sucked off and frozen at -20°C until assayed.

Measurements. Plasma kallikrein activity, prekallikrein, and functional kallikrein inhibition values were determined with the chromogenic peptide substrate S-2302 (Kabi, Stockholm, Sweden) using an automated enzyme analyzer (Cobas Bio, Hoffmann La Roche, Basel, Switzerland) as previously described (20). The chromogenic substrate S-2251 (Kabi) was used to determine plasminogen, plasmin and antiplasmin values. The values of prekallikrein, kallikrein inhibition, plasminogen, and antiplasmin are given as percentages of standard plasma pool values. Antithrombin III values were assayed with the chromogenic substrate S-2238 (Kabi) as previously described (20). Plasma total protein was measured with a routine technique (21).

HK and its degradation products were determined by SDS PAGE followed by immunoblotting according to standard procedures (22, 23). The blot was washed, quenched, and incubated with rabbit antiserum against human HK. Bound antibodies were finally detected with alkaline phosphatase-conjugated goat antibodies against rabbit IgG. The developed immunoblots were scanned and the area under the curve for each band was used as a measure of the relative amount of native kininogen or degradation product (24).

The project was approved by the ethical committee of the hospital, and informed consent was obtained from the parents

of the babies. Statistical differences between the groups were tested by a two-tailed t test. A p value < 0.05 was considered statistically significant.

RESULTS

Oxygenation requirements and PaO_2 . In Figure 1, FiO_2 in the three patient groups is given until d 4 after birth. The mean initial FiO_2 was 0.91 (SD 0.09) in group 1 (RDS surfactant) and 0.94 (SD 0.09) in group 2 (RDS patients without surfactant) (NS). On d 2 (24 h after surfactant instillation in group 1 babies), the FiO_2 was, however, significantly lower in surfactant-treated RDS patients (mean 0.38, SD 0.08) compared with nontreated RDS patients (mean 0.82, SD 0.08) ($p < 0.04$). Mean FiO_2 remained lower in the surfactant group also at d 3 (mean 0.43, SD 0.22) compared with nontreated RDS babies (mean 0.80, SD 0.13) ($p < 0.05$). On d 4, mean FiO_2 in surfactant-treated babies was 0.42 (SD 0.07) compared with 0.63 (SD 0.18) in nontreated RDS babies (NS).

The mean FiO_2 in the controls was initially 0.26 (SD 0.04), ranging between 0.21 and 0.30, significantly lower than in both of the RDS groups. After 48 h, eight of the nine controls breathed room air.

PaO_2 was initially 7.4 (SD 2.0) kPa in the surfactant-treated group (group 1) versus 10.1 (SD 4.1) kPa in group 2 (NS). During the study, mean PaO_2 in the RDS patients with and without surfactant treatment were not statistically different and varied between 6.5 and 10.1 kPa.

Plasma prekallikrein, kallikrein, and kallikrein inhibition. Plasma prekallikrein values are given in Figure 2. There were no differences in prekallikrein values between the surfactant-treated (group 1) and nontreated RDS (group 2) babies during the study. In the first plasma sample (d 1), the mean prekallikrein values were 22 (SD 6)% in group 1 ($p < 0.005$ versus controls), 21.5 (SD 5.0)% in group 2 ($p < 0.0015$ versus controls), and 32 (SD 8)% in the control group (group 3). Twenty-four h later (d 2), there was still no difference between the two RDS groups, but the differences between these two groups and the controls were even larger, with a mean of 20 (SD 5)% in group 1 ($p < 0.0002$), 21 (SD 6)% in group 2 ($p < 0.0005$), and 37 (SD 7)% in controls. The next 2 d prekallikrein values increased steadily and parallelly in all groups, reaching at d 4 a mean of 31 (SD 6)% in group 1 ($p < 0.05$ versus controls), 27 (SD 4)% in group 2 ($p < 0.02$ versus controls), and 47 (SD 15)% in controls.

Fig. 1. FiO_2 in three groups of patients the first 4 d after birth. RDS + S, RDS patients treated with a single dose of natural surfactant 5 min after the reading at d 1. RDS, RDS patients not treated with surfactant. CTR, premature babies without RDS who served as controls. Mean and SD are given as range.

Fig. 2. Plasma prekallikrein values as the percentage of standard plasma pool values the first 4 d after birth. *RDS + S*, RDS patients treated with a single dose of natural surfactant 5 min after the reading at d 1. *RDS*, RDS patients not treated with surfactant. *CTR*, premature babies without RDS who served as controls. Mean and SD are given as range.

Fig. 3. Plasma kallikrein inhibition (*KKI*) values as the percentage of standard plasma pool values the first 4 d after birth. *RDS + S*, RDS patients treated with a single dose of natural surfactant 5 min after the reading at d 1. *RDS*, RDS patients not treated with surfactant. *CTR*, premature babies without RDS who served as controls. Mean and SD are given as range.

The plasma kallikrein activity was low but stable, varying between 8 and 10 U/L in the two groups of RDS babies and between 7 and 12 U/L in the controls during the first 4 d after birth. No significant differences between the three groups were found.

Plasma kallikrein inhibition values increased in all the three groups during the study period (Fig. 3). There were no differences between groups 1 and 2 throughout the observation period. Group 1 babies had, however, significantly lower values than controls at d 2 ($p < 0.02$) and 4 ($p < 0.05$), whereas group 2 patients had values not significantly different from controls.

Proteolysis of HK. To study proteolytic activity in more detail, HK (band I) and its degradation products (bands II and III) were measured in RDS patients receiving surfactant (group 1) and compared with the controls at d 1 and 2. The mean total amount of kininogen (sum of bands I, II, and III) measured as area under

the curve was 5 081 (SD 2 327) in group 1 and 8 222 (SD 3 612) in controls at d 1 (NS, $p < 0.09$). At d 2, the values were 4 250 (SD 3 993) versus 13 279 (SD 19 000) (NS), respectively.

Band I values were significantly lower in group 1 patients at d 1 (mean 2 022, SD 1 365) than in controls (mean 4 370, SD 2 203) ($p < 0.04$). Band III (Fig. 4) values were equal in groups 1 and 3 at d 1. In group 1 babies, there was a dramatic decrease from a mean value of 1 163 (SD 530) to 150 (SD 34) ($p < 0.0025$) from d 1 to d 2. In controls, the amount of band III remained stable.

Figure 5 shows a SDS-PAGE immunoblot of HK and its degradation products in one RDS patient treated with surfactant the first week after birth. Band III, which is the most important indicator of HK proteolysis, was clearly present at d 1. After d 5 it disappeared completely.

Figure 6 demonstrates scanning of HK and its degradation products in the same patient at d 1 (left) and d 2 (right). It can be seen how band III is clearly present the first day and that the amount decreases until the next day. This indicates a reduced proteolysis of HK from d 1 to d 2.

These data strongly indicate that the rate of proteolysis of HK was slowed down from d 1 to d 2 in RDS patients.

The fibrinolytic system and antithrombin III. The results of the measurements of plasminogen, plasmin, and antiplasmin at d 1–4 in the three groups of patients are given in Table 1. There were no differences between the three groups except with regard

Fig. 4. The change in proteolysis of HK from d 1 to d 2 in babies with RDS treated with a single dose of surfactant (*RDS + S*) compared with premature babies without RDS serving as controls. Rate of proteolysis is illustrated by the relative amount of degradation product detected as band III. *AUC*, area under the curve. SD is given as range. *, $p < 0.0025$ RDS + S at d 1 vs d 2.

Fig. 5. Immunoblot of HK at d 1 to 7 after birth in one patient with RDS treated with a single dose of surfactant. Band I (molecular mass 149 000 D) represents HK, band II (molecular mass 104 000 D) and band III (molecular mass 99 000 D) represent proteolytic breakdown products of HK. Band III is clearly present at d 1. At d 6 and 7, it has completely disappeared.

Fig. 6. Scanning of the three bands derived from HK at d 1 and 2 in the same RDS patient as in Figure 4. Band III clearly is diminished from d 1 to d 2, demonstrating reduced proteolytic conversion of HK.

Table 1. Data from fibrinolytic system for first 4 d after birth*

	Day			
	1	2	3	4
Plasminogen (%)				
Group 1	31 (10)	31 (6)	36 (10)	38 (9)
Group 2	31 (10)	31 (9)	34 (9)	34 (11)
Group 3	32 (15)	37 (11)	39 (14)	50 (20)
Plasmin (U/L)				
Group 1	10 (7)	15 (9)	22 (17)	10 (7)
Group 2	15 (9)	21 (16)	27 (18)	29 (23)
Group 3	15 (12)	25 (15)	18 (9)	12 (8)
Antiplasmin (%)				
Group 1	58 (13)	67 (10)	72 (9)	75 (9)
Group 2	40 (27)	43 (26)†	48 (29)	42 (26)
Group 3	62 (23)	75 (13)	82 (19)	90 (22)

*Group 1, RDS patients treated with porcine surfactant; Group 2, RDS patients not treated with surfactant; Group 3, premature control babies without RDS. Plasminogen and antiplasmin are given as the percentage of standard pooled plasma values. Mean values and SD (numbers in parentheses) are given.

† $p < 0.03$, RDS vs controls.

to plasma antiplasmin values, which at d 2 were significantly lower in the group 2 RDS babies than in controls.

Antithrombin III values were low compared to pool plasma values from normal adults, but there was neither any difference between the groups nor any significant change during the study (data not shown).

Plasma total protein. In control babies, plasma total protein

at d 1 and 2 were 39.6 (SD 1.8) g/L and 41.5 (SD 3.1) g/L, respectively. Plasma total protein was not determined in these babies later on. Plasma total protein did not differ between the two RDS groups, and the mean value was 35.3 (SD 3.4) g/L at d 1, which is significantly lower than values found for control babies ($p < 0.04$). The next 3 d, plasma total protein concentrations in RDS patients were stable, with mean values ranging between 37 and 38 g/L.

DISCUSSION

In the present study, we have demonstrated that plasma prekallikrein values were significantly lower in babies with severe RDS compared with control babies without RDS the first days after birth. These data are basically in accordance with our previous findings (6). During the first day of life, mean plasma prekallikrein in both groups of RDS patients was 63% of the values found in premature control patients without RDS. Although the plasma total protein was decreased in RDS babies as well, this reduction was, in mean, only 11% compared with control values. The differences in prekallikrein values found between the groups in this study is therefore probably not due to a general decreased protein synthesis in the liver in RDS babies. On the contrary, the data indicate that the plasma kallikrein-kinin system is activated in RDS.

The low HK levels found at d 1 in RDS patients, together with the rapid decrease in the amount of its degradation products (band III) from d 1 to d 2, as found in this study, represent the first direct proof that the plasma kallikrein-kinin system is activated in RDS. Rescue treatment with porcine surfactant does not seem to enforce or prevent this activation or influence the fibrinolytic system either. Whether or not prophylactic surfactant treatment inhibits activation of the plasma kallikrein-kinin system is not known and should be worthwhile to investigate.

Judging by plasminogen values and plasmin activity, the fibrinolytic system is not activated in RDS, in contrast to findings by Suzuki (25).

It has been previously demonstrated that plasma prekallikrein is transformed to kallikrein in a series of other pathophysiologic conditions such as septicemia, multitraumatized patients with multiple organ failure, and the adult RDS (1–5, 17). On the basis of the present data it may therefore be tempting to speculate that RDS has some features in common with "posttraumatic" diseases such as those listed above (1–5). It is known that RDS may be aggravated and precipitated by shock, hypothermia, and acidosis (26, 27). To what extent activation of the plasma kallikrein-kinin system influences the clinical development in patients with RDS is, however, uncertain.

In conclusion, we have found lowered plasma prekallikrein and kallikrein inhibition values in babies with RDS. The first day after birth, the total amount of HK is reduced in these patients compared with premature babies not having RDS. Furthermore, the rate of proteolytic degradation of HK is reduced in RDS babies from the first to the second day of life. We have therefore documented that the kallikrein-kinin system is activated in RDS. Rescue treatment with one dose of natural porcine surfactant does not seem to influence such an activation.

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