α_1 -Antitrypsin and α_1 -Acid Glycoprotein Levels in the Cord Blood and Amniotic Fluid of Infants with Respiratory Distress Syndrome

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Summary

α_1 -Antitrypsin, a proteinase inhibitor, was previously shown to be decreased in the cord blood and elevated in the amniotic fluid of infants with RDS. The low cord α_1 -antitrypsin in RDS may be the result of either decreased production or increased catabolism or loss of the protein by the fetus.

To determine whether there is a specific preferential decrease in α_1 -antitrypsin in the cord blood of infants with RDS, we have compared the reduction in blood levels of α_1 -antitrypsin with that of a low molecular weight protein which has no antiproteinase activity, α_1 -acid glycoprotein (mol wt = 41,000). If a molecular sieve type of leakage is basic mechanism responsible for the low α_1 -antitrypsin in the cord blood of infants with RDS, then α_1 -acid glycoprotein might be decreased at least to a comparable degree in the cord blood. Both proteins were measured by the radial immunodiffusion method in 36 cord blood specimens, 13 of which had matching amniotic fluids which were also studied.

There was a significant lowering of the cord blood α_1 -acid glycoprotein in preterm infants with RDS (12.4 ± 1.3) compared to those without RDS (21.1 ± 4.3) ($P < 0.05$). When the same specimens were analyzed for α_1 -antitrypsin, the difference between preterm infants with RDS and those without RDS did not reach statistical significance ($P = 0.12$).

These findings support the hypothesis the serum proteins are decreased in RDS mainly because of diffuse capillary leakage.

Speculation

The results of this study suggest that *in utero* hypoxia or other factors associated with low Apgar scores and abnormal blood gases result in increased capillary permeability. A detectable decrease in the concentration of one or more cord serum proteins, especially those of low molecular weight, may be a sensitive indicator of fetal distress and increased capillary permeability.

α_1 -Antitrypsin, a proteinase inhibitor, has been shown to be decreased in the cord blood (14) and elevated in the amniotic fluid of infants with RDS (16). However, other proteins, such as plasminogen (2), and antiproteinases, including serum elastase inhibitor (6) and α_2 -macroglobulin (5), also tend to be low in the blood of infants with RDS compared to normal preterm infants.

The cord α_1 -antitrypsin could be low in RDS because of either decreased production or increased catabolism of the protein by the fetus, including size-related molecular sieving through immature or leaky capillaries. Its low molecular weight of 55,000 may be an important factor. However, small size is not the only consideration since hyaline membranes in the alveoli in infants with RDS are largely derived from blood proteins, and many large and small proteins are found in the membranes (7, 9).

Nevertheless, to determine whether there is a preferential de-

crease in α_1 -antitrypsin in the cord blood of infants with RDS, we have compared the blood levels of a low molecular weight protein which has no antiproteinase activity, α_1 -acid glycoprotein (mol wt = 41,000), with the level of α_1 -antitrypsin (15). If a molecular sieve type of leakage is the basic mechanism responsible for the low α_1 -antitrypsin in cord blood of infants with RDS, then α_1 -acid glycoprotein should be comparatively decreased to at least a comparable degree in the cord blood, assuming a constant synthetic rate for the two proteins.

MATERIALS AND METHODS

Thirty-six cord blood specimens, matching amniotic fluids from 13 of these, and 6 additional amniotic fluids were collected and stored at -20° until the study was performed. Thirteen of the cord bloods and 5 of the amniotic fluids were from infants who subsequently developed RDS. The range of gestational age was from 25-36 weeks. Consecutively born non-RDS infants served as controls. They were unselected except for being age-matched to RDS infants. Specimens of cord blood, amniotic fluid, and maternal blood were collected during labor from six uncomplicated full term pregnancies. Eleven additional maternal bloods obtained during premature labor were also analyzed.

α_1 -Antitrypsin and α_1 -acid glycoprotein levels were measured by the radial immunodiffusion method of Mancini, using plates from Behring Diagnostics (Department of Hoechst Pharmaceuticals, Inc., Somerville, NJ). Measurements on adult sera were performed on undiluted specimens. In study cord blood and amniotic fluid α_1 -acid glycoprotein, each well was filled five times, and we waited until the fluid in the well had been absorbed before refilling.

Total serum protein was estimated by refractometry. Statistical analyses were made utilizing the Student *t*-test.

RESULTS

The cord blood α_1 -antitrypsin levels at various gestational ages in infants with or without RDS are shown in Figure 1. There was not a significant relationship between the levels of α_1 -antitrypsin and gestational age in the 23 "normal" infants ($r = -0.125$; $P > 0.1$). The same statement holds true for α_1 -acid glycoprotein ($r = 0.05$; $P > 0.1$) (Fig. 2). The mean normal α_1 -antitrypsin in full term infants was 179 ± 29 mg/100 ml and the α_1 -acid glycoprotein was 20.0 ± 2.6 mg/100 ml. In normal preterm infants (without RDS) the mean α_1 -antitrypsin was 229 ± 22 mg/100 ml and α_1 -acid glycoprotein was 21.1 ± 4.3 mg/100 ml (Table 1).

The changes in these two proteins, α_1 -antitrypsin and α_1 -acid glycoprotein, in the serum of preterm infants with RDS are also indicated in Table 1. The difference in α_1 -antitrypsin between preterm infants with RDS and without RDS did not reach statistical significance ($P = 0.12$). However, there was a significant

Fig. 1. The cord blood α_1 -antitrypsin was not significantly related to gestational age in the 23 "normal" infants ($r = -0.125$, $P > 0.1$) or in those infants with RDS ($r = -0.24$, $P > 0.1$). Infants with RDS are depicted by open circles (○).

Fig. 2. The cord blood α_1 -acid glycoprotein was not related to gestational age in the 23 "normal" infants ($r = 0.05$, $P > 0.1$) or in those infants with RDS ($r = -0.38$, $P > 0.1$). ○, infants with RDS.

lowering of the serum α_1 -acid glycoprotein in preterm infants with RDS compared to those without RDS (12.4 ± 1.3 vs. 21.1 ± 4.3). The ratio of α_1 -acid glycoprotein over total protein was not significantly different in infants with or without RDS, which indicates that the total serum protein level was not decreased to the same degree as was α_1 -acid glycoprotein in infants with RDS.

In amniotic fluid, the mean α_1 -acid glycoprotein for 6 full term infants was 6.4 ± 1.8 mg/100 ml, which was somewhat lower than that for 13 preterm infants ($93. \pm 2.8$ mg/100 ml). This difference did not reach statistical significance due to the small sample size ($P > 0.05$).

When the infants in this study were analyzed according to 5-min Apgar scores, those with a score of 6 or less did not show statistically different levels of α_1 -antitrypsin or α_1 -acid glycoprotein levels in cord blood from infants with an Apgar score greater than 6.

The mean maternal blood levels of α_1 -antitrypsin and α_1 -acid glycoprotein were 414.9 ± 34.4 mg/100 ml and 33.7 ± 2.8 mg/100 ml, respectively.

DISCUSSION

Significantly lowered levels of serum total protein (4, 13), plasminogen (2) α_2 -macroglobulin (5), and α_1 -antitrypsin (14) have been found in RDS when large numbers of infants have been studied. It has not been clear whether these proteins were low because of decreased production, increased catabolism or because of nonspecific leakage into tissues, including the fetal lung.

Attention has been focused on infants with low levels of the antiproteinase α_1 -antitrypsin in RDS because of its known relationship to lung disease in older children and adults. Fierer *et al.* (7) have suggested that a lowered α_1 -antitrypsin may be of specific importance in the etiology of hyaline membranes since the protein is found within the alveoli in the majority of newborn infants with hyaline membranes.

El-Bardeesy *et al.* (5) have reported that there were significant decreases in cord α_1 -antitrypsin and α_2 -macroglobulin levels in infants with RDS. However, the lowering of albumin and total protein levels were not statistically significant. The fact that we found low levels of α_1 -acid glycoprotein but others have not found low levels of albumin might be due to a relatively rapid synthesis of albumin by the liver which could better compensate for losses into the lungs and interstitial tissues than in the cases of α_1 -antitrypsin or α_2 -acid glycoprotein.

Singer *et al.* (16, 17), however, have suggested that the low serum proteins in RDS may simply reflect a loss of many proteins into the pulmonary alveoli. It was postulated that the smaller the molecular size of the protein, the greater the loss, and the more likely there is a depletion of that protein in the serum. They suggested that if another serum protein of similar or lower molecular weight than the relatively small α_1 -antitrypsin were studied simultaneously in the cord blood of infants with RDS, it might be possible to answer the question of simple molecular sieving versus selective depletion of the antiproteinase, α_1 -antitrypsin. To resolve this problem, we have compared the cord serum level of a non-proteinase inhibitor α_1 -acid glycoprotein (mol wt = 41,000) to the

Table 1. Cord α_1 -antitrypsin and α_1 -acid glycoprotein in preterm infants with and without RDS

	RDS	No RDS	P value
No.	13	17	
Gestational age (week)	30.6 ± 0.8^a	31.9 ± 1.0	>0.5
α_1 -Antitrypsin (mg/dl)	191 ± 21.2	229 ± 22.0	0.12
Total protein (g/dl)	3.72 ± 0.32	3.94 ± 0.15	>0.25
α_1 -Antitrypsin/total protein (mg/g)	57.7 ± 11.8	57.8 ± 4.9	>0.5
α_1 -acid glycoprotein (mg/dl)	12.4 ± 1.3	21.1 ± 4.3	<0.05
α_1 -Acid glycoprotein/total protein (mg/g)	3.62 ± 0.63	5.28 ± 1.0	0.09

^a Mean $\pm$ SEM.

Fig. 3. The panel on the left is a schematic representation of the pores in a capillary wall such as might be found in full term infants or those with *in utero* growth retardation. Pulmonary capillaries are thought to be mature in most infants with normal lecithin/sphingomyelin (L/S) ratios. The right panel represents an immature capillary with increased numbers of pores as probably occurs in fetal immaturity, hypoxia, or RDS. This is associated with increased leakage of most serum proteins but predominantly those of lower molecular weight.

level of α_1 -antitrypsin (mol wt = 54,000) in preterm infants with or without RDS.

α_1 -Acid glycoprotein is a serum protein without antiproteinase activity which is synthesized in human fetuses as early as 32 days of gestation (9). Its normal level in adults is reported as 55–140 mg/100 ml (15). However, we found that the maternal blood level was 33.7 ± 2.8 mg/100 ml from 17 pregnant mothers. Also the level found in a serum pool from 12 healthy nonpregnant adults in our laboratory was 48 mg/100 ml. The serum level of this protein is known to be somewhat depressed in pregnancy, but increased in cancer, pneumonia, rheumatoid arthritis, postoperative periods, and in the active stage of ulcerative colitis (1, 12, 15). It is speculated that it has a regulatory role in the formation of striated collagen and in binding steroid hormones in the circulation (3).

The important finding from this study is that a nonproteinase inhibitor α_1 -acid glycoprotein was significantly lowered in infants with RDS compared to age-matched preterm infants without RDS. α_1 -Antitrypsin was also decreased in the infants with RDS but in this small group the reduction was not statistically significant ($P = 0.12$). Since earlier and larger studies have shown a significant lowering of α_1 -antitrypsin in infants with RDS (5, 14), it appears that the lowering of serum proteins in RDS is either nonspecific or is related to molecular size but not to antiproteinase activity. These findings support the hypothesis that serum proteins are decreased in RDS mainly because of diffuse capillary leakage.

RDS appears in preterm infants with an immature surfactant system. It is also likely that these infants with RDS have immaturity of other systems, including capillaries. Infants with RDS have characteristic increased tissue edema compared to normal infants. Thus, the immature fetus which is prone to develop RDS, probably has increased capillary leakage prior to birth, and this may be aggravated by *in utero* hypoxia or other complications of pregnancy (schematically represented in Fig. 3). There is no evidence at present of a selective depletion of α_1 -antitrypsin in infants who develop RDS. Careful investigation of the biosynthesis, catabolism, and maternofetal transfer of various serum proteins during the perinatal period are needed to thoroughly understand the dynamic changes which occur in infants with RDS.

CONCLUSION

α_1 -Antitrypsin, a proteinase inhibitor, is known to be decreased in the cord blood of infants with RDS. To determine whether there is a specific preferential decrease, α_1 -antitrypsin (54,000 mol wt) and α_1 -acid glycoprotein, which is a nonproteinase inhibitor with similar molecular weight (41,000), were measured by the radial immunodiffusion method in 13 cord blood specimens of preterm infants with RDS and in 17 age-matched non-RDS control specimens.

There was a significant lowering of the cord blood α_1 -acid glycoprotein in RDS ($P < 0.05$), which in this study was greater in degree than the lowering of the cord α_1 -antitrypsin ($P = 0.12$).

These findings support the hypothesis that serum proteinase inhibitors are decreased in RDS because of diffuse capillary leakage.

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