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Antiactivated *factor X*
antithrombin III
fetal hypercoagulability
idiopathic respiratory distress sydrome

Circulating Anticoagulants in the Newborn: Relation to Hypercoagulability and the Idiopathic Respiratory Distress Syndrome

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Extract

Cord blood from 106 high risk and 68 normal deliveries was studied for hypercoagulability and levels of the circulating anticoagulants, antithrombin III (AT-III) and antiactivated *factor X* (anti-Xa). Table I shows that normal, term, appropriate for gestational age (AGA) infants showed a deficiency at AT-III when compared with normal children and adults ($P < 0.001$). Preterm (Pr) AGA infants had a lower level of AT-III than term (T) AGA infants ($P < 0.001$). The lowest levels of AT-III were seen in infants who developed idiopathic respiratory distress syndrome (IRDS); 66% of all infants with an AT-III time of 18 sec or less (mean ± SEM AT-III value for T-AGA infants, 37 ± 2.8) developed IRDS. All except three of the infants (Fig. 1) with IRDS and/or low AT-III levels were offspring of mothers in the high risk category of premature labor or third trimester bleeding. When the thrombelastogram was used as a measurement of whole blood coagulability, T-AGA infants showed hypercoagulability when compared with adults and children ($P = 0.025$). The infants with the most hypercoagulability were offspring of the groups of severe erythroblastosis fetalis, third trimester bleeders, and premature labor.

As seen in Table I and Figure 2, anti-Xa activity is slightly lower in the Pr-AGA group than in adults ($P < 0.01$), but the level of anti-Xa does not correlate well with the development of IRDS.

Speculation

The susceptibility of the ill newborn infant to thrombotic, hemorrhagic, and necrotic pathologic lesions may be related to a deficiency of a potent, naturally occurring anticoagulant, antithrombin III. This AT-III, like α-1-antitrypsin, is a proteinase inhibitor. Both of these substances are severely deficient in infants with IRDS. The therapeutic implications of replacement of these proteins in groups of infants at risk to develop IRDS and hemorrhagic-thrombotic lesions remains to be explored.

Introduction

The concept of hypercoagulability of the cord blood in preterm infants who develop the idiopathic respiratory distress syndrome has been described (19). Hypercoagulability or shortening of the clotting time of fetal whole blood has been noted by others and can be considered a paradoxical finding in light of the physiologic deficiencies of certain coagulation factors (II, VII, IX, X, XI, XII) and platelet functional defects seen in the neonate (11, 21). The cause of hypercoagulability may be attributed to deficiencies of the naturally occurring anticoagulant system. The results of the following study did indicate a previously undescribed deficiency of a potent physiologic anticoagulant, antithrombin III (AT-III), the neonate. In addition, infants who developed IRDS uniformly showed a severe deficit of AT-III activity in the cord blood.

Materials and Methods

Subjects

Obstetrical patients at the University of Colorado Medical Center (1971-1972) and the University of Southern California-Los Angeles County Medical Center (May-July, 1972) were classified into high risk categories. These categories included (1) pre-eclampsia and eclampsia, (2) diabetes mellitus, (3) third trimester bleeders (abruptio placenta, placenta praevia), (4) primary premature labor (no complicating cause), (5) Rh sensitization with erythroblastosis fetalis (EBF), (6) fetal distress, and (7) normal term pregnancy. These patients were followed to delivery by a member of the research team. Immediately on delivery of the infant the umbilical cord was double clamped in two places, divided between the clamps, and given to the team member who obtained venous samples by needle puncture. Cord blood samples were obtained on *106 high risk infants; 37 of these were delivered by caesarean section*, the rest vaginally. Cord blood from 68 term infants of appropriate weight for gestational age from normal pregnancies were used as normal control subjects (this included 20 elective repeat caesarean sections).

Venous blood from 15 additional infants with severe IRDS during the first 6-24 hr of life was studied. No cord blood samples were available for this group.

The infants were cared for in the nurseries of the hospitals noted above. All infants were classified according to gestation age after the recommendations of Battaglia and Lubchenco (4).

The idiopathic respiratory distress syndrome was defined as tachypnea, chest retractions, and grunting in an infant lasting at least 12 hr and associated with a chest x-ray compatible with hyaline membrane disease. Thirteen infants with mild respiratory distress (lasting less than 48 hr) might also be considered to have "transient tachypnea of the newborn."

Methods

Blood was drawn from the cord segments into plastic syringes by a two-syringe technique. Citric acid-citrate anticoagulant was placed in the syringes in an appropriate amount for an estimated hematocrit of 50% except in the severe EBF patients for whom an estimate of 25% was used.

Thrombelastograms were done on recalcified citrated whole blood: the measurement of the r + k value (clotting time) was expressed in minutes. The antithrombin III (AT-III) and antiactivated factor X (anti-Xa) assays were performed on platelet-free plasma obtained after centrifugation of the whole blood. The AT-III assay was done within 8 hr (stored at 4°) and the anti-Xa assay was done on frozen samples within 4 weeks (stored at -20°).

The AT-III assay was done exactly as described by von Kaulla and von Kaulla (29) except that the plasma was defibrinated (56°, 15 min) and used in place of serum:

Defibrinated citrated plasma (0.4 ml)
+
Bovine thrombin, 15 units (incubated at 37° for 6 min)
↓
Mixture (0.2 ml)
+
Bovine fibrinogen, 1% (0.1 ml)

Time for clot

The shorter the clotting time the less the activity of AT-III. A "blank" of buffered saline instead of plasma has a 5 sec clotting time.

The anti-Xa assay was done according to the method of Biggs et al. (5)

Defibrinated plasma
+
Activated factor X (incubated at 37° for 10 min)
↓
Mixture assayed for residual factor X activity

The percentage of inactivated factor X is proportional to the anti-Xa activity; the values were expressed as percentage of anti-Xa.

Preliminary experiments showed that most of the Xa was inhibited by normal adult or cord plasma after a 10 min incubation; therefore, in order to conserve plasma, a single 10 min incubation time was used for the test samples.

Results

As there was no significant difference between the AT-III levels in caesarean section (mean = 36.2) and vaginally (mean = 37.8) delivered T-AGA infants ($P > 0.1$) or in the thrombelastogram findings, the data was pooled for analysis.

As shown in Table I, the normal T-AGA infants showed a deficiency of AT-III when compared with normal children and adults ($P < 0.001$). Preterm AGA infants had a lower level of AT-III than term AGA infants ($P < 0.001$). Preterm infants who developed IRDS showed a mean AT-III time of 12.9 sec; preterm infants without IRDS had a mean AT-III time of 24.7 sec ($P < 0.001$). The most striking deficiency of AT-III was seen in the infants who developed IRDS; these markedly low levels of AT-III were seen both in cord blood and venous blood in the first 24 hr of life. Figure 1 shows the cord blood AT-III levels in the group of preterm infants plus other infants developing IRDS. All but one of the infants with IRDS had an AT-III value lower than the range for values in the normal T-AGA babies. A few normal preterm infants had such low levels of AT-III, but 66% of all infants with an AT-III value of 18 sec or below developed IRDS.

In contrast, as seen in Table I and Figure 2, the level of anti-Xa does not correlate well with the development of IRDS, although anti-Xa activity is lower in the Pr-AGA group than in the adult and older children groups ($P < 0.01$).

In regard to the high risk infant groups, all except three of the infants with IRDS and/or low (18 sec or less) levels of AT-III were in the primary premature labor or third trimester bleeder group. The exceptions were a Pr-AGA infant (wt 1,800 g) with disseminated intravascular coagulation and a Pr-AGA infant (wt 1,240 g) with a normal outcome. Both of these infants were in the pre-eclampsia group and neither developed IRDS. The third infant (wt 2,920 g) was the Pr-LGA offspring of a class C diabetic mother; this infant developed mild IRDS.

The thrombelastogram was used as a measurement of whole blood coagulability. T-AGA infants were hypercoagulable when compared with *adults and children* ($P = 0.025$). *Even more hypercoagulable were the* groups of severe EBF, third trimester bleeder, and premature labor offspring (see Table I). Although most of the infants in the latter groups had abnormally low AT-III times, only about one-fourth of the infants had r + k times less than the range of term-AGA infants. Therefore, precise correlation of short clotting times (hypercoagulability) and low AT-III times in individual infants was not possible.

Discussion

A review of the recent literature suggests that the newborn infant is susceptible to thrombotic and necrotic pathologic lesions. A not uncommon lesion of the sick infant, intraventricular hemorrhage, has been related pathologically to subependymal infarction of the brain (26, 27). Hemorrhagic necrosis of the adrenal gland is occasionally seen as a complication in the ill preterm infant (7). Renal vessel thromboses are well known to occur in the infant of the diabetic mother (24, 31). Thrombotic complications of indwelling venous and arterial catheters have been shown with alarming frequency (22, 30). The neonate is also susceptible to disseminated intravascular coagulation (2, 6, 12). The fetal lung appears to be especially prone to thrombotic and fibrin lesions (3, 25). A recent report (14) suggests that necrotizing enterocolitis may also represent a susceptibility to hemorrhagic necrosis.

Hypercoagulability of fetal and neonatal blood has been documented previously (15, 19, 33). Hypercoagulability, or the measurement of shortening of the whole blood clotting time, is a clinical laboratory measurement and by itself is not necessarily related to thrombotic tendencies in the subject. The observations reported here confirm the studies of Markarian et al (19) which showed extreme cord blood hypercoagulability as well as a resistance to heparinization in those infants who developed IRDS (20). Studies of clotting factors and platelet function in the neonate reported previously (11) support hypocoagulability rather than hypercoagulability. However, blood coagulability in man may be influenced by deficiencies of naturally occurring anticoagulants (8, 23, 28). At least three such anticoagulants have been characterized: AT-III (1, 5, 29), anti-Xa (5, 32), and antiactivated factor XI (10). The AT-III deficiency noted above probably contributed to the hypercoagulable state seen in the neonate but is not necessarily the only reason for the shortening of the whole blood clotting time or the increased susceptibility to thrombotic lesions. Indeed, the measurement of whole blood coagulability used here (thrombelastogram) reflects the influence of the complex procoagulant system, the anticoagulant system, and noncoagulant factors such as viscosity, acid-base balance, and pharmacologic agents. Therefore, it was not surprising that hypercoagulability frequently did not correlate with low AT-III levels.

The relation of low levels of AT-III in the cord blood to the development of IRDS is especially intriguing. The data shown in Figure 1 indicate that a low cord blood AT-III level is associated with a higher incidence of IRDS, although an occasional normal preterm infant can have a low AT-III. Indeed, all infants studied to date who have developed IRDS have AT-III clotting times of 18 sec or less. Antithrombin III is a proteinase inhibitor with a specificity for thrombin; it is an alpha globulin with a molecular weight of about 65,000 (13). Another proteinase inhibitor, alpha-1-antitrypsin, has been shown very recently to be deficient in blood of infants with IRDS (9, 16, 17). The relation of these related substances to the pathogenesis of IRDS is not known at present. Currently available data do not permit one to interpret these low levels of proteinase inhibitors as practical aids in the diagnosis and management of IRDS.

The finding of only slightly low levels of anti-Xa activity in the neonate was surprising. Others (32) have suggested that AT-III and anti-Xa are similar and, possibly, identical proteins. However, there may be two types of anti-Xa (18), and it is possible the method used here was only sensitive to one type or not sensitive enough to determine a true deficiency. These possibilities remain to be explored.

Summary

The hypercoagulability of fetal and neonatal blood may be related to a deficiency of a naturally occurring anticoagulant called antithrombin III. AT-III is especially low in the cord blood of those infants who develop IRDS. Another anticoagulant, antiactivated factor X, is also reduced in the neonate, but the low level does not correlate well with the incidence of IRDS or prematurity. Low activity of AT-III in cord blood was most often seen in the high risk pregnancy groups of spontaneous premature labor and third trimester bleeders.

References and Notes

1. Abildgaard, U., Fagerhol, M. K., and Egeberg, O.: Comparison of progressive antithrombin activity and the concentration of three thrombin inhibitors in human plasma. Scand. J. Clin. Lab. Invest., 26: 349 (1970).

2. Altstatt, L. B., Dennis, L. H., Sundell, H., Malan, A., Harrison, V., Hedvall, G., Eichelberger, J., Fogel, B., and Stahlman, M.: Disseminated intravascular coagulation and hyaline membrane disease. Biol. Neonatorum, 19: 227 (1971).

3. Ambrus, C. M., Ambrus, J. L., Weintraub, D. H., Foote, R. J., Courey, N. G., and Niswarder, K. R.: Thrombolytic therapy in hyaline membrane disease. Thromb. Diath. Haemorrh. (Suppl.) 47: 259 (1971).

4. Battaglia, F. C., and Lubchenco, L. O.: A practical classification of newborn infants by weight and gestational age. J. Pediat., 71: 159 (1967).

5. Biggs, R., Denson, K. W. E., Akman, N., Barrett, R., and Hadden, M.: Antithrombin III, antifactor Xa, and heparin. Brit. J. Haematol., 19: 283 (1970).

6. Boyd, J. R.: Disseminated fibrin thromboembolism among neonates dying within 48 hours of birth. Arch. Dis. Childhood, 42: 401 (1967).

7. DeSa, D. J., and Nicholls, S.: Haemorrhagic necrosis of the adrenal gland in perinatal infants: a clinico-pathologic study. J. Pathol., 106: 133 (1972).

8. Egeberg, O.: Inherited antithrombin deficiency causing thrombophilia. Thromb. Diath. Haemorrh., 13: 516 (1965).

9. Evan, H. E., Keller, S., and Mandl, I.: Serum trypsin inhibitory capacity and the idiopathic respiratory distress syndrome. J. Pediat., 81: 588 (1972).

10. Forbes, C. D., and Ratnoff, O. D.: Studies on plasma thromboplastin antecedent (factor XI), PTA deficiency and inhibition of PTA by plasma: Pharmacologic inhibitors and specific antiserum. J. Lab. Clin. Med., 79: 113 (1972).

11. Hathaway, W. E.: Coagulation problems in the newborn infant. Pediat. Clin. N. Amer., 17: 929 (1970).

12. Hathaway, W. E., Mull, M. M., and Pechet, G. S.: Disseminated intravascular coagulation in the newborn. Pediatrics, 43:233 (1969).

13. Heimburger, N., Haupt, H., and Schwick, H. G.: Proteinase inhibitors of human plasma. In: H. Fritz and H. Tschesche: Proceedings of the International Research Conference on Proteinase Inhibitors, Chapt. 1. (Walter de Gryter, Berlin, 1971).

14. Hopkins, G. B., Gould, V. E., Stevenson, J. K., and Oliver, T. K.: Necrotizing enterocolitis in premature infants. Amer. J. Dis. Child., 120: 229 (1970).

15. Koch, F.: Bluteiweissuntersuchungen beim Neugeborenen unter Berucksichtigung der Gerinnungsstoerungen. Z. Geburtsh. Beilageheft, Bd. 159: 142 (1962).

16. Kotas, R. V., Fazen, L. E., and Moore, T. E.: Umbilical cord serum trypsin inhibitor capacity and the idiopathic respiratory distress syndrome. J. Pediat., 81: 593 (1972).

17. Makram, W. E., and Johnson, A. M.: Serum proteinase inhibitors in infants with hyaline membrane disease. J. Pediat., 81: 579 (1972).

18. Marciniak, E., and Tsukamura, S.: Two progressive inhibitors of factor Xa in human blood. Brit. J. Haematol., 22: 341 (1972).

19. Markarian, M., Githens, J. H., Rosenblut, E., Fernandez, F., Jackson, J. J., Bannon, A. E., Lindley, A., Lubchenco, L. O., and Martorell, R.: Hypercoagulability in premature infants with special reference to the respiratory distress syndrome and hemorrhage. I. Coagulation studies. Biol. Neonatorum, 17: 84 (1971).

20. Markarian, M., Lubchenco, L. O., Rosenblut, E., Fernandez, F., Land, D., Jackson, J. J., Bannon, A. E., Lindley, A., Githens, J. H., Martorell, R.: Hypercoagulability in premature infants with special reference to the respiratory distress syndrome and hemorrhage. II. The effect of heparin. Biol. Neonatorum, 17: 98 (1971)

21. Mull, M. M., and Hathaway, W. E.: Altered platelet function in newborns. Pediat. Res., 4: 229 (1970).

22. Neal, W. A., Reynolds, J. W., Jarvis, C. W., and Williams, H. J.: Demonstration of thrombi on umbilical catheters. Pediatrics, 50: 6 (1972).

23. Nesje, O. A., and Kordt, K. F.: Hypoantitrombinemi som arsak til mesanterialvenetrombose. Nord. Med., 83: 367 (1970).

24. Oppenheimer, E. H., and Esterly, J. R.: Thrombosis in the newborn: comparison between infants of diabetic and nondiabetic mothers. J. Pediat., 67: 549 (1965).

25. Sanerkin, N. G., Edwards, P., and Jacob, J.: Pulmonary thromboembolic phenomena in the newborn. J. Pathol. Bacteriol., 91: 569 (1966)

26. Towbin, A.: Central nervous system damage in the human fetus and newborn infant. Amer. J. Dis. Child., 119: 529 (1970).

27. Towbin, A.: Cerebral intraventricular hemorrhage and subependymal matrix infarction in the fetus and premature infant. Amer. J. Pathol., 52: 121 (1968).

28. von Kaulla, E., Droegemueller, W., Aoki, N., and von Kaulla, K. N.: Effect of estrogens on postpartum hypercoagulability and antithrombin III activity. Amer. J. Obstet. Gynecol., 113: 920 (1972).

29. von Kaulla, E., and von Kaulla, K. N.: Antithrombin III and disease. Amer. J. Clin. Pathol. 48: 69 (1967).

30. Wigger, H. J., Bransilver, B. R., and Blanc, W. A.: Thromboses due to catherization in infants and children. J. Pediat., 76: 1 (1970).

31. Woodward, J. R., Patterson, J. H., and Brinsfield, D.: Renal artery thrombosis in newborn infants. Amer. J. Dis. Child., 114: 191 (1967).

32. Yin, E. T., Wessler, S., and Stoll, P. J.: Biological properties of the naturally occurring plasma inhibitor to activated Factor X. J. Biol. Chem., 246: 3703 (1971).

33. Zillizcus, H., Ottelin, A. M., and Mattsson, T.: Blood clotting and fibrinolysis in human fetuses. Biol. Neonatorum 10: 108 (1966).

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