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New neonatal problems of blood coagulation and fibrinolysis

I. The change of plasmin inhibitor levels in the newborn infant.

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Blood coagulation and the lysis of the resulting thrombi are mainly governed by two principles: The levels of the enzymes and of the corresponding inhibitors involved. The most important fibrinolytic enzyme of the blood is plasmin. It circulates as plasminogen in the blood and is converted to the active protease on requirement (Fig. 1 a).

The conversion is catalysed by plasminogen activators like Urokinase, which are localized in the vessel wall.

Several protease-inhibitors of the human blood are involved in limiting the plasmin activity to the physiologically reasonable extent (Fig. 1 b). This balance between activation and inhibition of plasmin is of particular interest in the newborn since adaptation to extrauterine life requires proper alveolar perfusion with blood, which is impaired by thrombus formation.

Therefore the activation of a sufficient amount of plasminogen is necessary in cases of hypercoagulation to remove the fibrin strands from the walls of the alveoli.

The present paper reports daily variations of the concentration of plasmin inhibitors in normal newborn infants and in newborns who developed pulmonary hyaline membrane disease.

In vitro studies of the influence of urokinase on the concentration of plasmin inhibitors are also reported.

Curriculum vitae

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1936 born in Sapporo.
1963 graduated from
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tric and neonatal consumption coagulopathy and blood
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Vth congress of international society of Haemostasis and
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Author of many publications on fibrinolysis in obstetrics
and neonatology with one monograph.

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and Thrombosis.

1 Materials and methods

Blood from 15 newborn infants was collected by puncture of the umbilical cord vein using a disposable syringe. For control blood from the cubital vein of normal adults was taken.

Fig. 1-a. The mechanism of fibrinolysis.

Fig. 1-b. The correlation between plasminogen-activator and plasmin-inhibitor in the control of fibrinolysis.

The samples were tested by thromboelastography (TEG). From each child or control person two samples were tested: One without and the other after addition of 2.5 units urokinase (MOCHIDA, Tokyo, Japan) per ml. The concentration of the plasmin inhibitors α_2 -macroglobulin, α_1 -antitrypsin and antithrombin III prior to and after the addition of urokinase was measured using M-partigen-plates (Behringwerke, Marburg, West-Germany).

2 Case report

Infant: M. I. ♀ 2380 g.
37 week premature delivery, SFD (Small for date baby).
APGAR score 5, shake test 1/2 (+); at birth actual pH 7.25.

Fig. 2. Detection of plasmin-inhibitor, (α_2 -macroglobulin, α_1 -antitrypsin, antithrombin III.) by M-partigenplates. (Behringwerke, Marburg, West-Germany.)

A catheter was inserted into the umbilical cord vein, and bicarbonate therapy etc. was initiated. Six hours after birth, the respiratory rate was increased to 80 or 90 per minute.

T.E.G diagram showed a possible hypercoagulability, and at that time α_1 -antitrypsin and antithrombin III, were decreased (Fig. 3).

Ten hours after birth severe cyanosis appeared followed by alveolar collapse and the child died.

During the course of events, we followed the change of T.E.G., Platelet aggregation, antithrombin III, α_1 -antitrypsin, α_2 -macroglobulin.

We noted that throughout the entire course very little change was seen in α_2 -macroglobulin, but the decrease of α_1 -antitrypsin and antithrombin III was remarkable.

At autopsy one found that the pulmonary hyaline membrane was complicated by pulmonary hemorrhage.

3 Results

13 out of 15 blood samples from normal newborn infants showed prior to and after addition of uro-

kinase thrombelastogram patterns characteristic for consumption coagulopathy (Fig. 5), the T.E.G. of blood samples from adults showed no such patterns.

Upon the addition of urokinase, infant blood showed a remarkable decrease of antithrombin III (AT III). Levels of AT III of 100% prior to urokinase addition dropped to 40% when urokinase was added.

The concentration of α_1 -antitrypsin was lowered to 70%, α_2 -macroglobulin did not change remarkably (Fig. 6).

The levels of α_2 -macroglobulin, α_1 -antitrypsin, and antithrombin III in the first days after delivery are shown in Fig. 4.

α_2 -macroglobulin does not change very much during this period and its concentration is of the range of normal adults. The concentration of α_1 -antitrypsin and antithrombin III did not reach even half the level of normal adults (Tab. I).

Fig. 3. Case. M. I. The variation of plasmin-inhibitor and TEG in the first hours after birth.

Fig. 4. Daily variations in the concentration of plasmin-inhibitors during early newborn period (32 cases).

Fig. 5. The change of thrombelastogram prior to and after addition of urokinase.

Tab. I. The change of plasmin-inhibitor (mg/dl) during newborn infant (32 cases).

days after birth	cord	1	2	3
α_2 -macroglobulin	243.6 $\pm$ 39.2	251.1 $\pm$ 32.6	331.1 $\pm$ 38.6	336.0 $\pm$ 39.8
α_1 -antitrypsin	105.4 $\pm$ 25.8	125.6 $\pm$ 23.6	164.8 $\pm$ 16.6	171.4 $\pm$ 18.4
antithrombin III	11.6 $\pm$ 3.2	12.5 $\pm$ 2.8	15.4 $\pm$ 3.6	15.6 $\pm$ 4.8
days after birth	4	5	6	7
α_2 -macroglobulin	336.0 $\pm$ 39.8	338.4 $\pm$ 46.5	346.6 $\pm$ 49.4	356.4 $\pm$ 52.6
α_1 -antitrypsin	176.2 $\pm$ 21.8	187.3 $\pm$ 28.4	187.8 $\pm$ 24.6	194.1 $\pm$ 26.2
antithrombin III	10.4 $\pm$ 3.6	10.6 $\pm$ 3.8	13.8 $\pm$ 2.6	17.4 $\pm$ 3.8

Fig. 6. The change of concentration of α_2 -macroglobulin, α_1 -antitrypsin, and antithrombin III.

4 Discussion

During the newborn period two main coagulatory complications may occur: disseminated intravascular coagulation (DIC) and respiratory distress syndrome (R.D.S). Newborns who show symptoms of R.D.S may be divided clinically as follows:

- (1) pulmonary hyaline membrane only.
- (2) hyaline membrane and lung hemorrhage.
- (3) lung hemorrhage only.
- (4) atelectasis.
- (5) atelectasis plus lung hemorrhage.
- (6) others.

By X-ray or from the clinical course alone, differential diagnosis of these cases is not always easy. Laboratory investigations could be helpful for such a differentiation. Thus during the newborn period, the plasmin inhibitors show a tendency of remarkable lowering and it may be surmised that such a lowering of the plasmin inhibitors may constitute an exceptionally great danger if activator is released.

Especially in the case of lung hemorrhage, fibrinolysis will be a possible complication, since the activator causes a severe pathological state in the lung which is intensified by the lowering of plasmin inhibitors. Reports dealing with the relationship between pulmonary hyaline membrane disease and fibrinolysis have been available since a long time.

However, GILTON [4] first pointed out that the main component of the hyaline membrane is fibrin.

Later, TAYLOR and ABRAMS [16] showed by their in vitro experiments that fibrinolysis is inhibited by lipo-protein which is a surface active substance.

The question arises concerning the nature of the fibrinolytic defect of the newborn. In 1961 LIEBERMAN [6] reported that among the newborn especially in premature babies, plasminogen concentration is very low. This was demonstrated by investigating the fibrinolytic activity of lung tissue on fibrin-plates.

He argued that pulmonary hyaline membrane disease is more likely caused by an inhibitor of fibrinolysis than by the lack of the activator. MIYAHARA [8] using human fibrinogen and KSCN

extraction of the lung investigated the lung tissue activator activity and reported that in cases of pulmonary hyaline membrane disease uncomplicated by pneumonia or lung hemorrhage, a comparatively low fibrinolytic activity was found. In 1968 BLEYL [1] and STARK [13] reported that disseminated intravascular coagulation (DIC) is an important cause of pulmonary hyaline membrane disease. They stated that the fibrin monomers which seeped into the alveoli are polymerized therein.

Recently the connection between pulmonary hyaline membrane, DIC and predisposition to hemorrhage has been discussed, and the relationship between R.D.S. and F.D.P has become known [14, 2, 5, 18].

As may be seen in our report [14], the amount of F.D.P. which is formed and the decrease of platelets, indicative of DIC, suggest the occurrence of a reactive fibrinolytic activity in the blood.

If the fibrinolytic activity of the lung tissue is lowered, how can we explain that fibrinolysis of the blood has been accelerated?

Before attempting to discuss the general theme of fibrinolysis in R.D.S., we will add one observation of MARKARIAN [7] who reported that the level of prothrombin and Factor X of premature babies

with R.D.S who survived was higher than of premature babies with R.D.S who did not survive. According to our study [14] which compared between R.D.S. with bleeding and R.D.S. without bleeding, there is a greater prolongation of prothrombin time and partial thromboplastin time in the cases with bleeding than in those without.

SAMARTZIS [11] reported that the serum plasminogen during the disease was low, and PHILIPP [9] presented similar data.

ABRAMS et al [16], however, found extremely high values of the blood activator in cases of hyaline membranes. He reasons that in cases of pulmonary hyaline membranes due to hypoxia, a small amount of tissue activator is released and enters the blood circulation to raise the values there.

Thus, in spite of extremely high concentrations of the activator, it is ineffective since little plasminogen is present.

The present work indicates that an increase of the activator level results in a remarkable lowering of α_1 -antitrypsin and antithrombin III.

The low level of inhibitors seems to work synergistically with the high value of the activator to produce coagulation disorders.

Especially the low level of antithrombin III could be the reason for the development of DIC.

Summary

"Hemorrhage in the newborn" has long been recognized as merely a result of vitamin K deficiency. However, it is also recognized that fibrinolysis, especially the correlation between the plasminogen-activator and plasmin-inhibitors, plays an important role in this disease during the neonatal period.

With this in mind, we compared thromboelastograms (TEG) from samples with and without urokinase (plasminogen-activator). In 13 out of 15 newborn infant blood-samples (prior to and after addition of urokinase) the thromboelastogram showed the pattern of a consumption coagulopathy.

The change in the concentration of plasmin-inhibitor during the neonatal period was also measured using α_2 -macroglobulin, α_1 -antitrypsin and antithrombin III with M-partigen-plates.

The value of α_2 -macroglobulin showed normal adult levels but the value of α_1 -antitrypsin and antithrombin III did not even reach half of the adult level.

During the newborn period, the plasmin-inhibitor shows a remarkable lowering tendency and it may be surmised that with such a lowering tendency, plasmin-inhibitor may constitute an exceptionally large handicap when the activator is working. This is especially true in the cases of lung hemorrhage since the activator arises from a severe pathological state in the lungs and in addition because this is complicated by the lowering of plasmin-inhibitor.

These results indicate that the low level of plasmin-inhibitors work synergistically with the high value of activator. The low level of antithrombin III could be the reason for coagulation disorders such as disseminated intravascular coagulation, (DIC).

Keywords: Fibrinolysis, plasmin-inhibitor, plasminogen-activator, thromboelastogram (TEG)

Zusammenfassung

Probleme der Blutgerinnung und Fibrinolyse beim Neugeborenen. I. Wechsel des Plasmin-Inhibitor-Spiegels beim Neugeborenen.

Die Haemorrhagien des Neugeborenen wurden für lange Zeit als ein Ergebnis eines reinen Vitamin-K Mangels angesehen.

Dann wurde festgestellt, daß die Fibrinolyse besonders die Beziehung zwischen plasminogen-aktivator und plasmin-inhibitor eine wichtige Rolle bei der Entwicklung dieses Krankheitsbildes spielt. Ausgehend von dieser Tatsache haben wir eine Reihe von Untersuchungen durchgeführt.

Mit dem Thrombelastgraph (TEG) wurden vergleichende Untersuchungen zwischen Proben mit und ohne Zusatz von Urokinase (Plasminogen-Aktivator) durchgeführt. Bei der Untersuchung von Neugeborenen-Blutproben wurde in 13 von 15 Fällen festgestellt, daß nach Zugabe von Urokinase sich eine Verbrauchskoagulopathie ent-

wickelt. In einer zweiten Versuchsreihe wurde die Konzentration des Plasminogen-Inhibitors während der neugeborenen Periode von α_2 -makroglobulin, α_1 -antitrypsin, Antithrombin III mit M-Partigen untersucht. Dabei zeigte es sich, daß α_2 -makroglobulin normale Erwachsenen-Werte erreichte, wohingegen für α_1 -antitrypsin und Antithrombin III nicht einmal die Hälfte der Erwachsenen-Werte erreicht wurde.

Diese Ergebnisse zeigen an, daß der niedrige Spiegel an Plasminogeninhibitoren synergistisch mit den hohen Aktivator-Werten wirken und Gerinnungsstörungen wie die DIC (Verbrauchskoagulopathie) auslösen.

Schlüsselwörter: Fibrinolyse, Plasmin-Inhibitor, Plasminogen-Aktivator, Thrombelastogram (TEG).

Résumé

Nouveaux problèmes de coagulation sanguine et de fibrinolyse en néonatalogie

I. Changement des taux d'inhibiteur de plasmine chez le nouveau-né

«L'hémorragie du nouveau-né» a été longtemps considérée comme une simple conséquence d'un manque de vitamine K. Or, on sait aussi que la fibrinolyse et, plus spécialement, la corrélation entre l'activateur de plasminogène et les inhibiteurs de plasmine, joue un rôle important dans cette maladie durant la période néonatale. Compte tenu de cela, on a confronté les thromboélastgrammes (TEG) d'échantillons avec et sans urokinase (activateur de plasminogène). Sur 13 des 15 spécimens sanguins de nouveaux-nés (avant et après l'addition d'urokinase) le thromboélastgramme a montré l'échantillon d'une coagulopathie de consommation. On a mesuré le changement dans la concentration d'inhibiteur de plasmine durant la période néonatale en utilisant des macroglobulines α_2 , de l'antitrypsine α_1 et de l'antithrombine III avec des plaques de partigène M. La

valeur de macroglobuline α_2 s'est révélée d'un taux adulte normal tandis que les valeurs de l'antitrypsine α_1 et de l'antithrombine III n'atteignent pas même la moitié du niveau adulte.

Durant la période néonatale, l'inhibiteur de plasmine accuse une tendance réductrice prononcée et il est à supposer qu'un tel inhibiteur de plasmine à tendance de baisse puisse représenter un handicap exceptionnellement lourd en cas de travail de l'activateur. Ceci est particulièrement vrai dans les cas d'hémorragie pulmonaire du fait que l'activateur est un état gravement pathologique survenant dans les poumons et, de plus, que ceci est compliqué par la baisse de l'inhibiteur de plasmine.

Ces résultats indiquent que le taux réduit des inhibiteurs de plasmine agit synergistiquement avec les valeurs élevées de l'activateur. Le taux réduit de l'antithrombine III pourrait être à l'origine de troubles de coagulation comme la coagulation intravasculaire disséminée (DIC).

Mots-clés: activateur de plasminogène, fibrinolyse, inhibiteur de plasmine, thromboélastgraphe (TEG).

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