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Thromboxane B_2 Production by Fetal and Neonatal Platelets: Effect of Idiopathic Respiratory Distress Syndrome and Birth Asphyxia

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Summary

To study the production of proaggregatory thromboxane A_2 (TxA_2) by fetal and neonatal platelets, blood specimens were collected from umbilical cords immediately after delivery at term ($n = 22$), from newborn infants during the first 10 days of life ($n = 85$), from infants between 1 and 3 months of age ($n = 14$), and from healthy adults ($n = 18$). The blood samples were allowed to clot spontaneously at +37°C for 60 min, and the concentrations of thromboxane B_2 (TxB_2), a stable metabolite of TxA_2, in the sera were measured by radioimmunoassay and expressed as nanograms of $TxB_2/10^6$ platelets.

Platelet TxB_2 generation in term infants at the age of 1 day (1.344 ± 0.253 ng/10^6 platelets, mean ± SE, $n = 9$) was higher than that in cord blood (0.634 ± 0.042 ng/10^6 platelets, $n = 22$), or in infants of 1–3 months of age (0.881 ± 0.099 ng/10^6 platelets, $n = 14$), or in adults (0.869 ± 0.062 ng/10^6 platelets, $n = 18$). Increase in TxB_2 generation following birth was seen already at the age of 1 h (1.076 ± 0.114 ng/10^6 platelets, $n = 9$). TxB_2 synthesis in preterm infants (1.032 ± 0.136 ng/10^6 platelets, $n = 10$) did not differ from that in term infants on the 1st day of life, and idiopathic respiratory distress syndrome had no effect on it (1.029 ± 0.079 ng/10^6 platelets, $n = 19$). Severe birth asphyxia was accompanied by reduced TxB_2 formation (0.564 ± 0.201 ng/10^6 platelets, $n = 7$).

The mode of delivery, the birth weight, and the sex of the infants were not related to TxB_2 production on the 1st day of life. Neither maternal pre-eclampsia nor epidural analgesia during labor affected neonatal TXB_2 generation. The bleeding time also did not correlate with TxB_2 formation. It is suggested that a rapid, but transient stimulation in TxA_2 synthesis after birth may contribute to the neonatal adaptation of vascular platelet function.

Abbreviations

IRDS, idiopathic respiratory distress syndrome
TxA_2, thromboxane A_2
TxB_2, thromboxane B_2
PGI_2, prostacyclin

The hemostatic mechanisms mature throughout gestation (2); nevertheless, the platelets of healthy term neonates are characterized by impaired aggregation and release reaction in response to physiologic stimuli (13, 17, 22). This platelet dysfunction may be important to apparently healthy preterm infants, since it is suggested to correlate with intracranial bleeding in these infants (16). In addition, in many neonatal disorders, such as birth asphyxia and IRDS, deficient platelet function may be clinically significant, since these conditions are frequently accompanied by a hemorrhagic tendency (6).

TxA_2, the main derivative of arachidonic acid in the platelets, is the most potent endogenous proaggregatory agent which also causes vasoconstriction (5). It is produced and released during platelet aggregation and rapidly converted to its stable metabolite TxB_2 (4, 5). Although the significance of TxA_2 in adults has been exhaustively studied (4), little is known about TxA_2 in the human fetus and neonate although birth causes profound adaptations in platelet vascular functions (3). We therefore studied platelet TxB_2 production in cord blood, in healthy newborn infants, and in infants with severe birth asphyxia and IRDS.

SUBJECTS AND METHODS

Subjects. Eighty-five infants were studied during the first 10 days of life (Table 1). Thirty-three term (37 weeks of gestation or more) and 20 preterm (less than 37 weeks of gestation) infants were healthy, whereas 25 preterm infants had IRDS, the diagnosis of which was based on the following criteria: grunting, retractions of the chest, cyanosis, reticulogranular pattern on the chest x-ray and need of ventilatory support for a period longer than 48 h. The birth weight, gestational age and Apgar scores of the distressed infants were comparable with those of the healthy preterm infants (Table 1). Seven infants (five term, two preterm) were severely asphyxiated at birth (Apgar scores of 3 or less at 1 min and 6 or less at 5 min, as well as in need of assisted ventilation for at least 10 min after birth) because of placental abruption ($n = 2$) and cord compression ($n = 5$). In addition, blood samples were collected from umbilical cords in 22 term deliveries immediately after placental expulsion. We also studied nine healthy term infants at the age of 1 h, 14 healthy infants between 1 and 3 months of age and 18 healthy adults.

Thrombocytopenia (platelet count under 100×10^9/liter), hyperbilirubinemia (serum total bilirubin > 170 μmol/liter), and maternal or neonatal ingestion of drugs known to interfere with prostaglandin synthesis indicated exclusion from the study. The study was approved by the local Committee of Ethics. An informed consent was obtained from the parents or subjects in every case.

Sampling and Analytical Methods. Neonatal blood samples (1–2 ml) were collected into dry tubes from the descending aorta via an umbilical arterial catheter or from a peripheral vein without stasis in connection with routine clinical monitoring. Similar blood samples from older infants and adults were taken by venipuncture. Cord blood was drawn from the umbilical cord

Table 1. *Clinical data of the healthy term and preterm infants and infants with IRDS or severe birth asphyxia**

	Gestational age (weeks)	Birth weight (g)	Apgar score	
			1 min	5 min
Term infants ($n = 33$)	39 (37–42)	3530 (1900–4490)	9 (6–10)	9 (7–10)
Healthy preterm infants ($n = 20$)	33.5 (27–36)	1880 (1180–2850)	7 (5–9)	9 (6–10)
Preterm infants with IRDS ($n = 25$)	34 (26–36)	2230 (1210–3370)	8 (4–9)	9 (6–10)
Infants with severe asphyxia ($n = 7$)	40 (32–41)	3530 (2190–3830)	2 (1–3)	4 (2–5)

* Median values are shown with ranges in parentheses.

vein immediately after expulsion of the placenta. Blood was allowed to clot at 37°C for 60 min, and the serum was then separated and the concentration of TxB_2 was measured by radioimmunoassay (20). The intra-assay and interassay coefficients of variation were between 6.7 and 9.7%, and 18.6%, respectively. The amounts of TxB_2 released into serum during spontaneous clotting correlate closely with those released during induced aggregation in platelet-rich plasma (20). To avoid the effect of individual variation in platelet counts, the results were expressed as nanograms of TxB_2 per 10^6 thrombocytes. The site of the sampling had no effect on the platelets' capacity to generate TxB_2 (aortal blood, 1.278 ± 0.207 *versus* venous blood, 1.191 ± 0.140 ng/10^6 platelets, $n = 8$).

Bleeding time was measured in nine healthy term and six preterm infants and in 15 preterm infants with IRDS during the 1st week of life, using the method of Ivy (14). Arterial P_{O_2}, P_{CO_2}, pH, and base excess were determined using routine laboratory methods.

Statistical Methods. The results were subjected to the analysis of variance and t testing modified by the Bonferroni method (21).

RESULTS

Platelet TxB_2 production increased from the fetal value by the age of 1 h and was at the age of 1 day higher than that in infants of 1–3 months of age or in adults (Table 2). TxB_2 on the first days of life did not differ either between the healthy term and preterm infants (Table 3) or between 27 and 29 weeks (1.040 ± 0.142 ng/10^6 platelets, $n = 6$; mean ± SE) and 33–36 weeks (0.961 ± 0.163 ng/10^6 platelets, $n = 6$) of gestational age. The platelets of asphyxiated infants generated reduced amounts of TxB_2 on the 1st day of life, whereas in IRDS the TxB_2 release did not differ from that in control infants (Table 3).

The bleeding time varied from 1.5 to 10.5 min and was not related to TxB_2 synthesis (Fig. 1).

There was no correlation between TxB_2 formation and the simultaneous arterial P_{O_2}, P_{CO_2}, pH, or base excess in infants with birth asphyxia or IRDS. Two asphyxiated infants had tendency to bleed (bloody tracheal and gastric secretions). One of them with a TxB_2 production of 1.250 ng/10^6 platelets subsequently died because of severe asphyxia, but no hemorrhages were found at autopsy.

The mode of delivery (vaginal delivery *versus* cesarean section), the birth weight and sex of the infants were not related to TxB_2 generation on the 1st day of life. Neither maternal preeclampsia ($n = 6$) nor epidural analgesia during labor ($n = 5$) affected neonatal TxB_2 formation on day 1.

DISCUSSION

It is clear from our results that platelet TxA_2 production is stimulated rapidly after birth. This stimulation lasts for 2–10 days, after which TxA_2 formation returns to the levels seen in the cord blood or in adults. Thus, it is evident that, in studies on the significance of TxA_2 in newborns, platelets from the neonate and not from the umbilical cord (18) should be used. In principle, our finding of similar TxB_2 generation in cord blood and adults matches well with the data of Stuart and Allen (18). Although

Table 2. *Platelet TxB_2 production (ng/10^6 platelets, mean ± SE) in cord blood in healthy term infants and in adults*

Age	n	Thromboxane B_2 (ng/10^6 platelets)
Cord blood	22	0.634 ± 0.042
Hour 1	9	1.076 ± 0.114*
Day 1	9	1.344 ± 0.253†
Days 2–10	15	0.936 ± 0.026
Months 1–3	14	0.881 ± 0.099‡
Adults	18	0.869 ± 0.062§

* $P < 0.025$.
† $P < 0.01$ in comparison with cord blood.
‡ $P < 0.05$.
§ $P < 0.025$ in comparison with the 1st day value.

Table 3. *Platelet TxB_2 production (ng/10^6 platelets, mean ± SE) in infants with severe birth asphyxia in healthy term and preterm infants, and in infants with IRDS*

	Thromboxane B_2 (ng/10^6 platelets)			
	n	Day 1	n	Days 2–10
Infants with birth asphyxia	7	0.564 ± 0.201*		
Term infants	9	1.344 ± 0.253	15	0.936 ± 0.026
Preterm infants				
Healthy	10	1.032 ± 0.136	14	0.908 ± 0.133
With IRDS	19	1.029 ± 0.079	13	1.141 ± 0.098

* $P < 0.05$ in comparison with the healthy term and preterm infants.

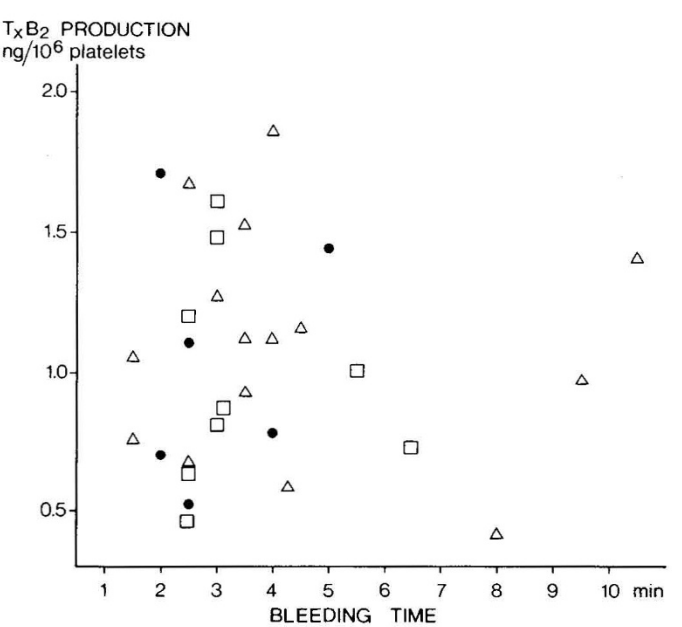

Fig. 1. Platelet TxB_2 production (ng/10^6 platelets) in relation to the bleeding time (min) in nine healthy term (□) and six preterm (●) infants and in 15 preterm infants with idiopathic respiratory distress syndrome (△).

they found decreased TxB_2 synthetase activity in cord blood platelets, the net TxB_2 synthesis was reported to be similar in fetal and adult platelets, because precursors of TxA_2 formation were increasingly available in fetal platelets (18).

With the data available, it is impossible to deduce the mechanisms behind the TxA_2 stimulation in newborns, as seen in this study, but a rapid rise in oxygen tension following the start of respiration may be a contributing factor. Oxygen, on the other hand, is the primary stimulus for the postnatal closure of ductus arteriosus (1, 3). Taking these data together, it does not seem unlikely that oxygen could promote ductal closure by stimulating the generation of the vasoconstrictive TxA_2 in neonatal platelets. It is, however, also possible that the increased TxA_2 synthesis after birth may compensate for the concomitantly enhanced vascular production of the anti-aggregatory and vasodilative prostacyclin (PGI_2) (10, 15), thus keeping these biologically important agents in balance.

It is well established that neonatal platelets are characterized by impaired aggregation (8, 13), which improves over the first weeks of life (11). The reason for this phenomenon is, however, unknown. We present here strong evidence suggesting that neonatal platelets already in the smallest preterm infants generate normal, or even increased amounts of TxA_2. Thus, defective synthesis of pro-aggregatory TxA_2 cannot explain the functional disturbances in neonatal platelets (8, 13). Furthermore, we show here for the first time that the platelets' capacity to generate TxA_2 is not related to bleeding time in healthy or distressed newborns, and a similar finding was recently reported from healthy adults (19). Factors other than TxA_2, perhaps changes in the membranes of the platelets (17), may account for the functional disturbances of neonatal platelets.

The infants with severe birth asphyxia had reduced platelet TxA_2 generation. In effect, their TxA_2 synthesis was of the same order as seen in the umbilical cord blood. The mechanisms by which asphyxia leads to inhibition of the TxA_2 formation are not known. However, as in fetal life, low arterial oxygen tension may be a contributing factor, but against this explanation speaks a lacking correlation between TxB_2 generation and postnatal arterial Po_2. The clinical significance of the decreased TxA_2 formation in asphyxiated infants remains unknown, although it may contribute to hemorrhages in these infants (7).

In view of reduced TxA_2 in birth asphyxia, it was curious that infants with severe IRDS, who often are hypoxemic, had normal TxA_2 in our study. The clinical signs of IRDS usually become manifest in the first few hours of life (12). The lag period with rather adequate oxygenation may be long enough to restore the TxA_2 biosynthesis to the normal neonatal level. It is, however, known that the production of the TxA_2 antagonist prostacyclin is increased during the acute phase of IRDS (9). Thus, the balance between TxA_2 and PGI_2 is shifted to a dominance of the anti-aggregatory PGI_2, which change may favor the manifestation of hemorrhages in IRDS. However, many other factors could also contribute to a bleeding tendency in distressed infants (7).

In conclusion, platelet TxA_2 production increases rapidly after birth in healthy term and preterm infants and in infants with IRDS. Such a rise is not seen in severe birth asphyxia.

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