Pediatr. Res. 15: 971–973 (1981)

blood
prostaglandin I_2
vitamin E

Deficiency of Plasma PGI_2-Like Regenerating Activity in Neonatal Plasma. Reversal by Vitamin E *In Vitro*

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Summary

Blood from full-term newborns was compared to adult blood for its ability to regenerate prostaglandin I_2 (PGI_2)-like activity from vascular tissue. The neonate possesses a markedly decreased ability to regenerate PGI_2 (0.10 ± 0.07 ng/mg vascular tissue) when compared to the adult (0.42 ± 0.12). This decreased activity was not due to the presence of an inhibitor in neonatal blood. The impaired ability of neonatal blood to regenerate PGI_2-like activity was related to its markedly decreased antioxidant potential and was corrected (0.34 ± 0.08 ng/mg vascular tissue) by the addition of Vitamin E *in vitro*. Plasma PGI_2-like regenerating activity had normalized by 3 to 5 months of age (0.41 ± 0.11 ng/mg).

Speculation

The neonate demonstrates a normal bleeding time despite concomitant impairment in platelet function. Our finding of a deficiency of plasma prostaglandin I_2 (PGI_2)-like regenerating activity may provide an explanation for the paradoxical normal neonatal bleeding time. Plasma PGI_2-like regenerating activity normalized by 3 to 5 months of age, at a time when platelet function also is no longer impaired. The physiologic impairment in platelet function observed in the neonate is thus a teleologic necessity, providing a compensatory safety mechanism to counteract the prothrombotic tendency induced by a deficiency of plasma PGI_2-like regenerating activity.

The only physiologic state associated with hypercoagulability and an increased susceptibility to thrombotic complications occurs in the neonatal period (1, 6). Various components of the hemostatic mechanism have been previously evaluated in the neonate in an attempt to elucidate the cause of this hypercoagulable state. Fluid phase coagulation factors are either normal (I, V, and VIII) or decreased (II, VII, IX, X, XI, and XII), and platelet function is well recognized to be impaired such that the hypercoagulable state cannot be ascribed to these changes (2, 6, 7, 8, 15). A deficiency of antithrombin III, the most important of the zymogen inhibitors, is present in the neonate (10). Inasmuch as familial antithrombin III deficiency predisposes affected individuals to thrombosis, neonatal hypercoagulability has been ascribed to the decrease in the level of antithrombin III that occurs neonatally.

A recent advance in understanding the dynamics of platelet-endothelial interaction has been the elucidation of the pattern of prostaglandin and thromboxane production in platelets and endothelial cells (14). Platelets produce thromboxane A_2 which is proaggregatory and prothrombotic, whereas endothelial cells produce prostacyclin or prostaglandin I_2 (PGI_2) which is antiaggregatory and antithrombotic. The balance of their production appears to play a major role in normal hemostasis. An imbalance in this critical interaction between the platelet and vessel wall can predispose to bleeding or thrombosis in certain pathologic states. For example, in the hemolytic uremic syndrome, Remuzzi *et al.* (18, 20) have demonstrated that the microangiopathic hemolytic anemia and microthrombosis appears to be related to a deficiency or total absence of plasma constituents necessary for normal PGI_2 synthesis by vascular tissue. We wish to report that normal neonates have a marked decrease of the plasma factor(s) necessary to stimulate vascular PGI_2 production. Moreover, we have demonstrated that the abnormality is reversible by the addition of an antioxidant, *i.e.*, vitamin E, to neonatal plasma.

MATERIALS AND METHODS

Blood samples were collected after informed consent from 11 normal adult controls aged 24 to 36 years who had not ingested any medication for 10 days before evaluation and from 11 normal full-term neonates. All infants weighed more than 2500 g and were born of healthy mothers after normal, full-term pregnancies and uneventful deliveries during which no drug recognized to affect platelet function had been administered. Immediately after delivery, clamps were placed on the umbilical cord, a 19-gauge needle was inserted into the umbilical vein, and blood was drawn into a plastic syringe. Blood samples were also obtained on eight infants aged 3 to 5 months.

Blood samples were mixed with 0.126 M trisodium citrate. The adult blood was anticoagulated by using 9 parts of blood to 1 part citrate. To adjust for the higher hematocrit of cord blood, the blood:citrate ratio in the latter experiments was 11:1. Platelet-free plasma was obtained by centrifugation of the citrated blood for 15 min at 1800 × *g*.

ASSESSMENT OF CAPACITY OF PLASMA TO STIMULATE VASCULAR PROSTACYCLIN OR PGI_2 ACTIVITY

The capacity of plasma (control adult *versus* neonate) to stimulate the generation of PGI_2-like activity was performed by the method of Remuzzi *et al.* (18–20) using "exhausted" human umbilical arterial rings obtained from normal, full-term deliveries. The umbilical arteries were thoroughly cleaned (200 to 400 mg wet weight), cut into fine rings, and kept in Hanks balanced salt solution (HBSS) without calcium (pH 7.4) at 0°C for no more than 60 min after removal. Rings (30 to 45 mg) were incubated with 120 μl of HBSS with calcium (pH 8.1) for 3 min at 22°C. Aliquots (30 μl) of the supernatant were added to 70 μl samples of normal platelet-rich plasma (300,000 platelets/μl), and the mixture was incubated for 1 min before the addition of adenosine diphosphate (Sigma Chemical Co., St. Louis, MO) at a final concentration of 3.0 μM. Prostacyclin-like activity was evaluated in a Payton dual-channel aggregometer by the bioassay described by Moncada *et al.* (13). Antiaggregatory activity was detected in all umbilical vessels evaluated. The umbilical arterial rings were then washed several times with HBSS (pH 7.4) until no antiaggregatory activity could be detected as evaluated by the above method. The exhausted vascular rings were then incubated for 30 min at 37°C with 120 μl plasma (control adult *versus* neonate) in each of 11

sets of experiments with incubations being performed in duplicate. Under these conditions, plasma from the normal control adults stimulated the exhausted vascular rings to generate prostacyclin-like or antiaggregatory activity. The amount of antiaggregatory activity of both the adult control and neonatal plasma was expressed as ng of PGI_2 per mg wet tissue by extrapolation from a dose-response standard curve obtained concomitantly with PGI_2 (The Upjohn Co., Kalamazoo, MI). PGI_2 activity was further characterized according to previous criteria (13). The activity was heat labile (*i.e.*, was lost by boiling for 0.25 min) and was unstable at room temperature (22°C for 20 min), and its generation was completely inhibited by 30-min incubations with either acetylsalicyclic acid (200 μM) or indomethacin (5 μg/ml). Mixing experiments were also performed in which 1 part neonatal plasma was incubated with 1 part adult control plasma, and the plasma PGI_2 regenerating activity of the resultant mixture was determined and contrasted to control plasmas diluted 1:1 with HBSS.

In six further paired experiments, vitamin E was evaluated *in vitro* for its ability to potentiate the effect of normal neonatal plasma to regenerate PGI_2-like activity. Vitamin E (dl-alpha-tocopherol in vehicle; Hoffman-LaRoche, Nutley, NJ) at a final concentration of 0.2 mg/ml, or the E vehicle (24) alone was added to neonatal plasma before incubation with exhausted umbilical arterial rings. After the incubation procedure previously outlined, the ability of neonatal plasma in the presence of either vitamin E or E vehicle was evaluated for its ability to generate PGI_2-like activity in the exhausted vascular tissue. PGI_2 regenerating ability was also assayed in duplicate from blood samples obtained from eight infants aged 3 to 5 months.

Plasma vitamin E was assayed by the method of Quaife *et al.* (17). Statistical comparison of results was made using the paired and unpaired Student *t* tests.

RESULTS

As depicted in Figure 1, adult platelet-poor plasma stimulated exhausted human umbilical arterial rings to produce 0.42 ± 0.12 (±1 S.D.) ng of PGI_2-like activity per mg wet weight of tissue. This value was significantly greater ($P < .001$) than the PGI_2-like generating ability of normal neonatal plasma (0.10 ± 0.07 ng/mg). The deficiency in plasma PGI_2 regenerating activity did not appear to be due to the presence of an inhibitor in neonatal plasma because appropriate correction (0.24 ± 0.06) was observed in the 1:1 mixing experiments. By 3 to 5 months of life, the PGI_2 regenerating ability of plasma had normalized (0.41 ± 0.11 ng/mg) and was similar to the values present in adult control plasma.

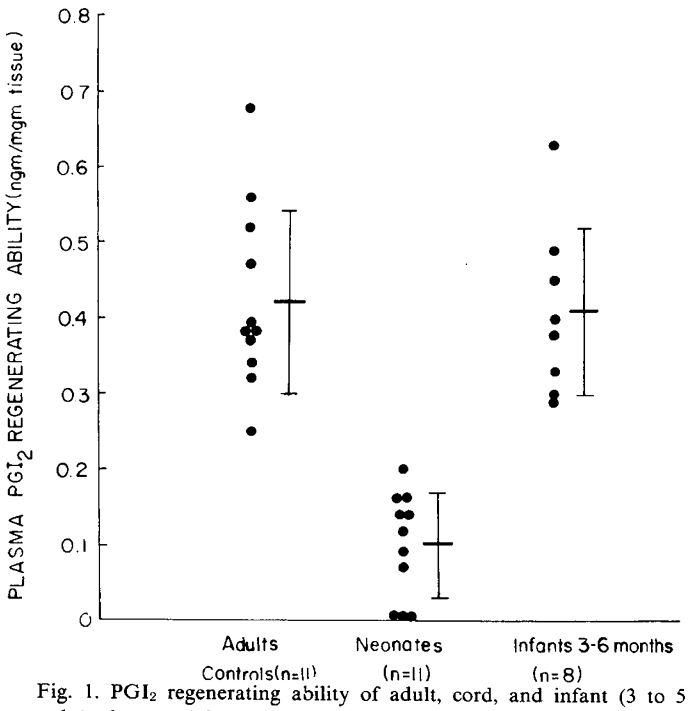

Fig. 1. PGI_2 regenerating ability of adult, cord, and infant (3 to 5 months) plasmas. PGI_2 regenerating ability is expressed as ng/mg wet weight of exhausted human umbilical arteries. Mean ± 1 S.D.

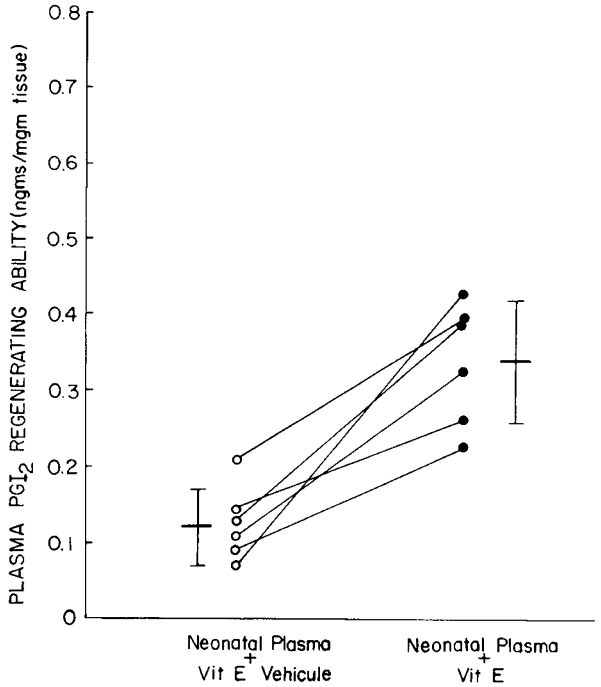

Fig. 2. PGI_2 regenerating ability of cord plasma in the absence of Vitamin E (○) and in the presence of 0.2 mg/ml of vitamin E (●). Values depicted are the results of six paired experiments. PGI_2 generating ability is expressed as ng/mg wet weight of exhausted human umbilical arteries. Mean ± 1 S.D.

Figure 2 depicts the results of the six further paired experiments in which vitamin E was evaluated *in vitro* for its ability to potentiate the PGI_2 regenerating capacity of normal neonatal plasma. In the presence of the vitamin E vehicle, cord blood regenerating activity was similar to that of cord blood alone (0.12 ± 0.05 ngm/mg). However, the addition of vitamin E *in vitro* to neonatal plasma significantly ($P < 0.01$) increased its ability to regenerate PGI_2-like activity (0.34 ± to 0.08 ng/mg). Mean plasma vitamin E levels in the 11 adult controls was 1.26 ± 0.56 mg/dl which was significantly higher than the values obtained in the neonates (0.28 ± 0.15 mg/dl).

DISCUSSION

It is recognized that neonates exhibit a transient thrombocytopathy. Hrodek (7, 8) observed a reduction in platelet factor 3 activity and availability and decreased platelet adhesiveness. The platelets of the newborn infant also demonstrate impaired aggregation to the physiologic agents adenosine diphosphate, epinephrine, collagen, and thrombin (2, 7, 8, 15). Impaired release of nonmetabolic platelet adenine nucleotides (3) and decreased platelet prostaglandin endoperoxide formation (22) have also been observed. However, in spite of these abnormalities, the neonate demonstrates a normal bleeding time (6). The bleeding time is the most sensitive *in vivo* test available to evaluate platelet and vascular function (9). Although quantitative assessment of the amount of blood lost through the bleeding time site has been advocated as a more subtle measure of platelet-vessel wall interaction, the marked aggregation abnormalities and impaired nucleotide release observed in the neonate should be of sufficient magnitude to cause a prolongation in their bleeding time. Thus, the finding in the neonate of a normal bleeding time has remained unexplained.

Prostaglandin biosynthesis by both the platelet and vascular endothelium appears to play a key role in normal and abnormal hemostasis. Various aggregating stimuli initiate platelet thromboxane A_2 biosynthesis by the liberation of arachidonic acid from platelet membrane phospholipids. The released fatty acid is then converted to the cyclic endoperoxides prostaglandins G_2 and H_2 in the presence of the enzyme cyclo-oxygenase. These endoperoxides are finally converted in the platelet to thromboxane A_2. A similar sequence of events occurs in the endothelium of the vessel wall with the final conversion of the cyclic endoperoxides to Prostacyclin of PGI_2 (14). Because thromboxane A_2 is proaggregatory and prothrombotic whereas PGI_2 is antiaggregatory and antithrombotic, a balance between the biosynthesis of these two opposing compounds plays a significant role in normal hemostasis. Most recently, in certain pathologic states associated with an increased thrombotic tendency, an imbalance has been observed in vascular-platelet prostaglandin production. In diabetes mellitus, both in the animal model (4) and in man (5, 21), increased platelet thromboxane A_2 production associated with a decrease in vascular PGI_2 production has been demonstrated. In the hemolytic uremic syndrome and in thrombotic thrombocytopenic purpura, the thrombotic tendency and hypercoagulable state appear to be related to a deficiency or absence of the ability of plasma to regenerate PGI_2 in exhausted vascular tissue (18, 20). We have demonstrated a similar abnormality in neonatal plasma. This finding of a decrease in the ability of neonatal plasma to regenerate vascular PGI_2 may provide an explanation for the presence of a normal bleeding time in the newborn, and together with the previously described (10) antithrombin III deficiency, explains his susceptibility to thrombosis.

Although the ability of neonatal plasma to regenerate PGI_2-like activity in exhausted vascular tissue is decreased when compared to adult plasma, endothelial cells derived from human umbilical cords have been demonstrated to produce prostacyclin or PGI_2 (11, 23). However, no comparative evaluation of PGI_2 production from neonatal vascular tissue as compared to activity in vessels from older infants or adults has been reported to date. The nature of the plasma factor regulating prostacyclin or PGI_2 activity has not been characterized at the present time. Our findings suggest that the decrease in activity is not due to a plasma inhibitory factor. The deficiency, however, appears related to the markedly decreased antioxidant potential of neonatal plasma, which in part appears due to the low levels of plasma vitamin E seen in the neonate. Most recently, Okuma *et al.* (16) have demonstrated that the vessels from vitamin E-deficient rats produced markedly decreased amounts of PGI_2, with a concomitant increase in lipid peroxidation products. After E repletion, vascular lipid peroxidation decreased, and PGI_2 activity returned to normal. Although we did not evaluate vascular thiobarbituric acid-reactive material as an indicator of lipid peroxidation, it is possible that the state of relative E depletion seen in the neonate causes increased lipid peroxidation of vascular tissue with the net result being a decrease in PGI_2 synthesis. The free radical scavenger vitamin E by inhibiting lipid peroxidation would enhance PGI_2 synthesis. Thus, the results of Okuma *et al.* are complementary to our findings.

Moncada *et al.* (12) have previously demonstrated that the generation of PGI_2 is strongly inhibited by 15 hydroperoxy-arachidonic acid (12). This observation has led them to suggest that inhibition of vascular PGI_2 by lipid peroxides could contribute to those diseases in which excessive lipid peroxidation occurs, such as atherosclerosis. Our finding that vitamin E when added to neonatal plasma *in vitro* increases its potential for vascular PGI_2 regeneration, together with the data of Okuma *et al.*, provides a possible rationale for the use of antioxidants in the prevention and treatment of atherothrombotic disorders.

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24. Emulphor EL-620, alcohol anhydrous, propylene glycol, benzyl alcohol, sodium acetate, glacial acetic acid, sodium chloride, disodium edetate, sodium hydroxide, and water (Hoffman-LaRoche, Inc., Nutley, NJ).

25. The author thanks Dr. John E. Pike (The Upjohn Company, Kalamazoo, MI) for his generous gift of PGI_2 and Ellen Blinder for technical expertise. Nancy Bovalino provided secretarial assistance.

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27. This research was supported by Research Grant 6-181 from the National Foundation—March of Dimes.
28. Received for publication September 3, 1980.
29. Accepted for publication November 6, 1980.

0031-3998/81/1506-0971$02.00/0

Printed in U.S.A.