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Contractile Activity of Neonatal Platelets

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ABSTRACT. Platelet contractile activity was evaluated by observation of tension development during isometric contraction of platelet-fibrin clots. Cylindrical clots were made with platelet-rich plasma obtained from cord blood or from adult controls. These clots were allowed to contract isometrically at 37° C while attached to a transducer to record tension development. The rate of tension development was dependent on platelet concentration but was equivalent for neonatal and adult platelet clots. Although abnormalities in neonatal platelet aggregation and secretion have been well documented the platelet functions required for clot contraction such as fibrin binding and actin-myosin interaction appear to be intact in neonatal platelets. (*Pediatr Res* 21: 293–295, 1987)

The transient physiologic alterations in the neonatal hemostatic system have been of interest for many years and of major clinical importance to those caring for sick and premature infants. The differences in clotting factor activity and fibrinolytic enzymes between cord blood and that of adult controls are well established (1, 2). Abnormalities in neonatal platelet function have been demonstrated using both cord blood and venous samples from newborn infants (3–6). These abnormalities include impaired aggregation responses to stimuli such as collagen, epinephrine, and ADP. Defective granule secretion has been described both as a decreased storage pool of ADP and as a faulty release mechanism. Decreased platelet factor 3 has also been described, suggesting that there may be differences between the platelet plasma membranes of adults and neonates. Mull and Hathaway (3) reported abnormal clot contraction in neonatal whole blood clotted with thrombin and observed after incubation at 37° C for 24 h. Clot contraction is dependent on the ability of activated platelets to bind fibrinogen or fibrin and to form an organized contractile cytoskeleton of actin and myosin (7, 8). Since these particular functions (fibrinogen binding and actin-myosin interaction) are also integral parts of the platelet aggregation response it is possible that defects in these mechanisms are responsible for the abnormalities in both platelet aggregation and clot contraction. The previous study of whole blood clot contraction (3) was a qualitative evaluation only. We have, therefore, studied tension development in plasma clots contracting under isometric conditions to quantify and compare contraction of clots made from cord blood and blood from adult controls.

MATERIALS AND METHODS

Cord blood was collected from the umbilical vein immediately after clamping the cord of 19 term infants (≥37 wk gestation) delivered by elective cesarian section. The blood was drawn through disposable needles into plastic syringes and immediately added to plastic tubes containing one volume citrate/citric acid/dextrose anticoagulant for nine volumes of blood. Mothers were in good general health with no history of aspirin ingestion. Blood was obtained by venipuncture into the same anticoagulant for 29 adult volunteers with no history of bleeding diathesis or aspirin and alcohol ingestion. Platelet-rich plasma was prepared by centrifugation of the citrated whole blood at 200 × *g* for 20 min at room temperature. Platelet counts were performed on the platelet rich plasma using a Coulter counter model ZBI (Hialeah, FL). Platelet-rich plasma was used to avoid possible confounding effects associated with differences in hematocrit among the samples. Some samples were studied in duplicate. Three adult donors were studied on more than one occasion.

Isometric measurements were performed using the technique of Cohen *et al.* (7) with modification. In brief, cylindrical clots were obtained by pouring 1 ml of platelet rich plasma into siliconized glass cylinders (5.0 × 0.6 cm ID) sealed at one end with parafilm. One U/ml bovine thrombin was added, the open end of the cylinder sealed with parafilm and the contents mixed gently by inversion. The plasma was allowed to clot for 9 min at 21° C, and the clot was poured into a Petri dish containing calcium-free Tyrode buffer at 2° C to inhibit contraction. The clot was tied at each end with cotton thread to stainless steel wire holders and immersed in a glass tissue bath containing chilled Tyrode buffer and surrounded by a water jacket. One holder was clamped in place and the other attached to an adjustable measuring/recording apparatus consisting of a Gould/Statham model UL5 micro-scale accessory, a UL3 unidirectional universal transducing cell, a SC1001 universal transducer readout, and a Kipp and Zonen BD 40 chart recorder. A preload of 78 mg was applied to straighten the clot and the clot was warmed by circulating 37° C water through the water jacket. The clot was allowed to contract for 60 min while tension generation was recorded. Regression analysis was done using version 5 SAS (Statistical Analysis System) and the University of Manitoba Computer Service on-line statistics.

RESULTS

The results of tension measurements were reproducible as shown by a variation of ±3% for samples studied in duplicate and ±9% for samples obtained for the same adult donor on two different occasions.

The rate of tension development in the plasma clots varied with platelet concentration for both newborns (Fig. 1*A*) and adults (Fig. 1*B*).

Analysis of the regression lines shows no significant difference suggesting that there is no difference in the rate of tension development in clots made from cord blood compared to clots made from adult blood.

DISCUSSION

The use of an isometric system for measuring clot contraction allows quantitative measurement of tension generation. The rate

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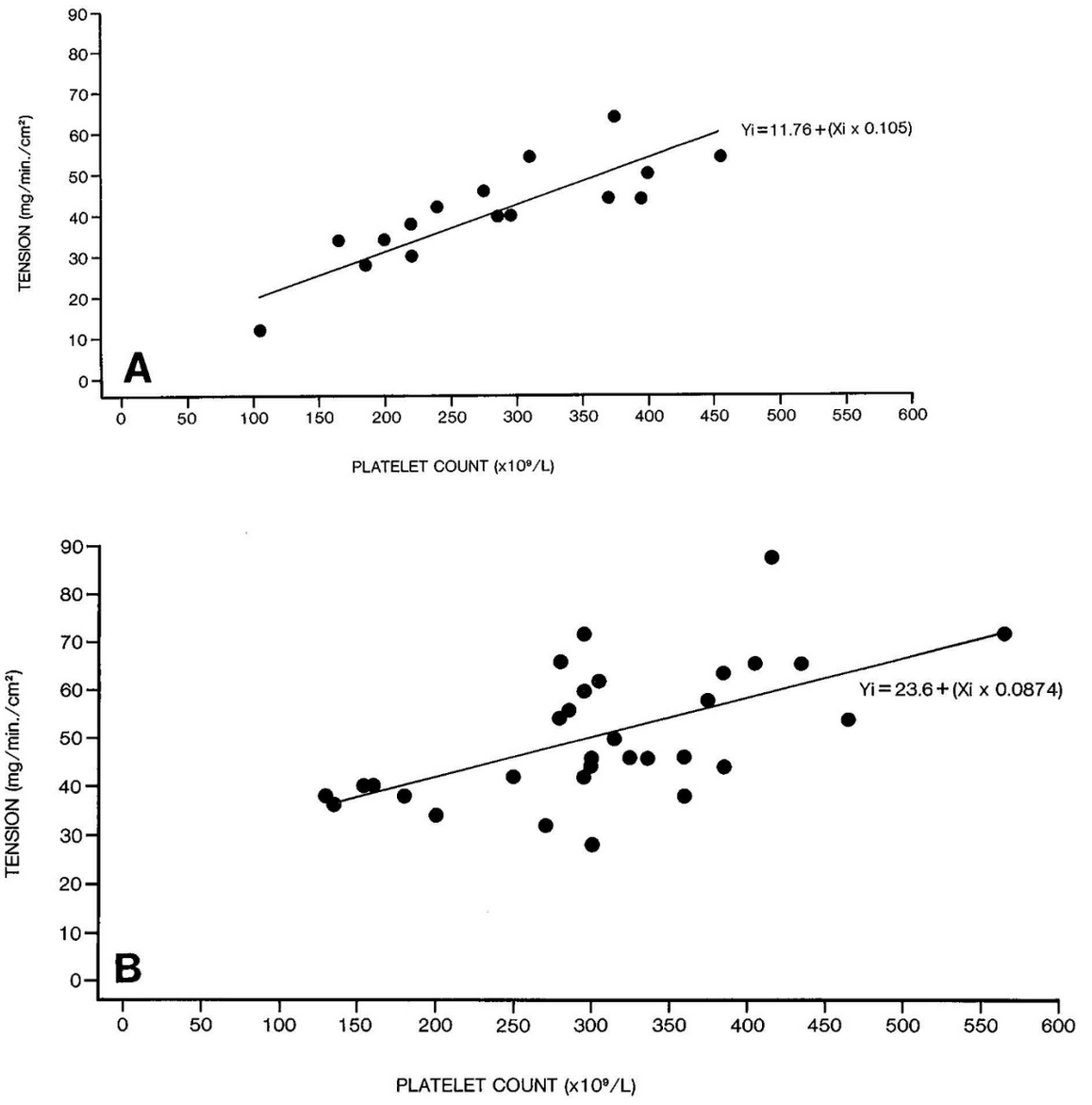

Fig. 1. The rate of tension development in thrombin-clotted platelet rich plasma varies with increasing concentrations of platelets. *A*, cord blood ($n = 19$); *B*, adult blood ($n = 32$). The equations describe the best line for each set of data.

of tension generation varies with platelet concentration as previously demonstrated by Cohen *et al.* (7). Tension has also been shown to depend on the presence of the platelet fibrinogen receptor (glycoprotein IIb/IIIa) and on an intact platelet contractile cytoskeleton (7). Our study demonstrates no difference in the ability of neonatal and adult platelets to facilitate clot contraction. These results suggest that the components of platelet function necessary for contractile activity are present in term infants (≥37 wk gestation). Although platelet aggregation studies were not performed on these cord blood samples, due to constraints of sample volume, multiple reports have confirmed abnormalities in neonatal platelet aggregation responses by well-established methodology (3–6). Therefore, this present study suggests that the previously described abnormalities of neonatal platelet aggregation are unlikely to be the result of defects in mechanisms shared by the aggregation and contraction processes, *i.e.* fibrin binding and actin-myosin interaction.

The only other evaluation of neonatal clot contraction by Mull and Hathaway (3) reported decreased retraction in 23 term infants based on simple observation of a whole blood clot incubated for 24 h. The discrepancy between their finding and our own may be due to a number of factors, including the crudity of the method available at that time compared to more precise measurement of tension generation now available. Platelet counts were not reported for infants or controls, and since contraction is dependent on this count, it is not possible to determine whether the two groups were comparable. Finally, the hematocrit is significantly higher in newborns than in adults and it is possible that this larger mass of red cells prevents the clot contracting to the same extent as one containing a lower concentration of red cells.

Clot contraction plays an important role in recanalization of thrombosed vessels by compacting the clot and facilitating clot lysis (9). Normal clot contraction by neonatal platelets is important in the face of other transient coagulation abnormalities which may predispose neonates, especially sick neonates, to thrombosis. These abnormalities include polycythemia, and decreased levels of antithrombin III (10), as well as iatrogenic causes

such as indwelling catheters in the umbilical artery. It appears that, despite other defects in neonatal platelet function, the mechanism for clot contraction is intact.

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