ORIGINAL ARTICLE

Platelet storage pool deficiency in Jacobsen syndrome

JAMES G. WHITE

Department of Laboratory Medicine and Pathology and Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA

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Abstract
Jacobsen syndrome and Paris-Trousseau Syndrome share similar congenital anomalies, thrombocytopenia, giant platelet alpha granules resulting from fusion of smaller organelles, and an 11q terminal deletion at 11q23.3. Similarities in the two cohorts have suggested that the Paris-Trousseau Syndrome is a variant of Jacobsen syndrome, or the same disorder. The present study has pointed out a significant difference between the two syndromes. Platelets from six patients with Jacobsen syndrome were markedly diminished in serotonin adenine nucleotide rich dense bodies, indicating the presence of platelet storage pool deficiency. Since platelet dense bodies are reported to be normal in size, number and distribution in the Paris-Trousseau Syndrome, the presence of platelet storage pool deficiency in six patients evaluated in the present study may distinguish the two disorders.

Keywords: Storage pool deficiency, Jacobsen syndrome

Introduction
Jacobsen syndrome is the classic genetic disorder involving chromosome 11 with a terminal deletion of 11q23 [1–7]. Typical congenital abnormalities include trigonocephaly, dysmorphic facies, cardiac defects, short stature, mental retardation and, in addition, ophthalmological, gastrointestinal and genitourinary problems, and thrombocytopenia.

In 1995, Breton-Gorius et al. [8] reported a condition with congenital dysmegal-karyocytopoiesis, thrombocytopenia, giant platelet alpha granules, together with clinical features and cytogenetic findings comparable to those in Jacobsen disorder, that they named the Paris-Trousseau syndrome [8–10]. The authors noted the genetic and clinical similarities, including thrombocytopenia reported in 47% of patients with Jacobsen syndrome, but apparently considered that the presence of giant platelet alpha granules distinguished the two syndromes. However, in 2001 Krishnamurti et al. [11] reported the presence of Paris-Trousseau syndrome platelets in a child with Jacobsen syndrome, and suggested that Paris-Trousseau syndrome was a variant of, or identical to Jacobsen syndrome. In 2003, the French workers presented 10 new cases of Paris-Trousseau syndrome [12]. Due to the clinical, hematologic and cytogenetic similarities between their cohort and Jacobsen syndrome, the authors concluded that their study demonstrated a clear overlap between the two conditions, as Krishnamurti et al. had reported [11].

Thus, the question whether there is any difference between Jacobsen syndrome and Paris-Trousseau syndrome remains to be answered. In 2000 Berry et al. [13] evaluated the frequency of dense bodies (DB) in platelets from two patients with Jacobsen syndrome. DB were absent from the platelets of one patient and markedly decreased (0.03 DB/platelet), in the other (normal: 4–8 DB/platelet). The report by Favier et al. [12] on 10 new patients with Paris-Trousseau syndrome indicated that the number, size and distribution of DB in platelets from their patients were normal.

The difference in findings regarding the presence or absence of platelet storage pool deficiency in the two syndromes suggested it might serve as a way of distinguishing them. This study has evaluated the frequency of DB in platelets from six patients with clinical and cytogenetic characteristics of
Jacobsen syndrome. Dense bodies were markedly decreased or absent in the platelets from all six patients.

Materials and methods

Patients

Blood from patients with Jacobsen syndrome was sent to us at room temperature by overnight express mail after informed consent by their parents. The 11q23 chromosomal deletion cytogenetic study had been carried out or was in process and later confirmed in all six individuals. Two of the patients were brothers. The other four patients were unrelated.

Blood samples

Blood samples were mixed after venepuncture with citrate-citric acid-dextrose, pH 6.5, in a ratio of nine parts blood to one part anticoagulant. Some blood samples were mixed with 100 mmol/liter EDTA in the same ratio. Platelet-rich plasma (PRP) was separated by centrifugation at room temperature for 20 min at 100 × g.

Fixation

Fixation of suspended platelets was accomplished by combining the sample with an equal volume of 0.1% glutaraldehyde in White’s saline (a 10% solution of a 1:1 mixture of: (1) 2.4 mmol/l NaCl, 0.1 mmol/l KCl, 46 mmol/l MgSO4 and 64 mmol/l Ca(NO3)2·4H2O; and (2) 0.136 mol/l NaHCO3, 8.4 mmol/l NaH2PO4 and 0.1 g/l of phenol red, pH 7.4 [14]. After 15 min the samples were centrifuged to pellets, washed and resuspended in 3% glutaraldehyde in the same buffer, resuspended and maintained at 4°C for 30 min, then sedimented to pellets. The supernatant was removed and replaced with either 1% osmic acid in Zetterquist’s buffer or 1% osmic acid in distilled water containing 1.5% potassium ferrocyanide for 1 h at 4°C. All samples were dehydrated in a graded series of alcohols and embedded in Epon 812. Thin sections cut from the plastic blocks on an ultramicrotome were examined unstained or after staining with uranyl acetate and lead citrate to enhance contrast. All examinations were made in a Phillips 301 electron microscope.

Preparation of platelet whole mounts

Small drops of PRP were placed on formvar-coated, carbon-stabilized grids, rinsed within 10–15 seconds with drops of distilled water, dried from the edge with pieces of filter paper and waved in the air to remove residual moisture [15]. The grids were inserted into the electron microscope without fixation or staining.

Results

Thin sections

Normal Platelets. The fine structure of normal platelets has been described in previous publications [16–19]. The discoid form of the resting cell is supported by a circumferential coil of microtubules lying just under the cell membrane (Figures 1 and 2).

Figure 1. Thin section of normal human platelets cut in cross section. Discoid form is supported by a circumferential coil of microtubules (MT). Many alpha granules (Gr), a few mitochondria, occasional dense bodies (DB) and numerous masses and single particles of glycogen fill the cytoplasm. Tortuous elements of the surface connected open canalicular system (OCS) are present as are channels of the dense tubular system. A giant granule (GGr) may be found in some normal platelets. Mag × 13 000.

Figure 2. Thin section of a normal platelet cut in the equatorial plane. A circumferential coil of microtubules (MT) just under the surface membrane supports the discoid form. Alpha granules (Gr), occasional dense bodies (DB), mitochondria and glycogen (Gly) are randomly dispersed in the cytoplasm. Elements of the tortuous open canalicular system (OCS) are also dispersed throughout this cell. Mag × 25 000.
The cytoplasm contains numerous alpha granules, a few mitochondria, and occasional adenine nucleotide and serotonin rich dense bodies. Present also are glycogen particles, rare Golgi complexes, and elements of two channel systems, the surface connected open canalicular system and the dense tubular system originating from endoplasmic reticulum in the parent megakaryocyte. The alpha granules are relatively uniform in size, but an occasional granule may be large (Figure 1). Many approach giant size if the blood remains unfixed for several days, or has been stored for longer periods of time [20].

Jacobsen Platelets. Thin sections of most platelets from patients with Jacobsen syndrome are similar in all respects to normal platelets. However, some of them contain giant spherical, oval, or irregular giant granules (Figures 3–8). We have shown these giant organelles derive by fusion of alpha granules [11] (Figures 6–8). The giant granules were present in platelets from all six patients with Jacobsen syndrome evaluated in the present study. The number present varied from 3 to 15–20%. The giant organelles were identical in appearance to those reported to be in platelets from patients with Paris-Trousseau syndrome and Jacobsen syndrome [8–12].

**Whole mounts**

Normal Platelets. DB are inherently electron opaque. Therefore, they are easily identified in unfixed, unstained whole mounts of normal platelets (Figures 9 and 10). Most dense bodies are spherical and easily distinguished from the chains and clusters found in normal and storage pool deficient platelets which are also inherently electron opaque [21, 22]. Some DB have long, whip-like tails, the origins of which remains uncertain (Figure 10). There are four...
to eight dense bodies per platelet in samples from normal subjects.

Jacobsen Platelets. The frequency of DB in platelets from all six patients with Jacobsen syndrome was strikingly less than the 4–8 DB/platelet observed in normal individuals (Figures 11–14). One patient had no dense bodies in the 100 platelets counted. Another had only 0.01 DB/platelet, and another 0.05 DB/platelet. The other three had slightly more (0.1 DB/platelet, 0.2 DB/platelet, and the last 0.09 DB/platelet). The numbers of DB are so low as to be virtually insignificant.

Discussion

Jacobsen syndrome [1–7] and Paris-Trousseau syndrome [8–10, 12] are very similar. They share the same clinical, hematological, and cytogenetic features. Both are caused by a terminal deletion of 11q23.3. Patients with both syndromes are thrombocytopenic in early life, and have giant α granule inclusions in their platelets. As a result, it seemed reasonable to conclude that Paris-Trousseau syndrome was a variant of Jacobsen syndrome, or the two disorders were the same [11].

The present investigation was stimulated by the possibility there may be some significant difference between the two disorders, and its characterization might lead to a better understanding of both conditions. Our group had noted in 2000 that dense bodies were nearly absent in two patients with Jacobsen syndrome [13] and suggested platelet storage pool deficiency was a feature of the disorder. The observation was supported by a second investigation reporting giant granules in Jacobsen syndrome platelets and a storage pool defect [23]. The present study has confirmed these earlier reports and
suggested that platelet storage pool deficiency may become a diagnostic feature. It is important because Grossfeld et al. [24] have found that even after platelet counts become close to normal in Jacobsen syndrome patients, they still may have markedly prolonged bleeding times. Thus, abnormal platelet function may cause bleeding problems, despite resolution of the thrombocytopenia. Recognition of platelet storage pool deficiency can lead to appropriate therapy, including platelet transfusion.

A major purpose of this study was to determine if there was a significant difference between patients with Jacobsen and Paris-Trousseau syndromes. The results demonstrated that platelets from patients with Jacobsen syndrome are markedly deficient in dense bodies indicating they are storage pool deficient. Studies by the workers describing the Paris-Trousseau syndrome have not indicated any deficiency in platelet dense bodies or the presence of platelet storage pool deficiency [8–10, 12]. Based on this significant difference, it may be reasonable to conclude Jacobsen and Paris-Trousseau syndromes are different disorders, despite their overlap.

References


