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Original article

A transmission electron microscopy study of anticoagulant-induced platelet vesiculation

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ABSTRACT

Platelet microparticles (PMPs) are small membrane fragments released from activated platelets in response to various stimuli. PMPs serve as biomarkers for several diseases and conditions and are useful tools for prognostic, diagnostic, and therapeutic purposes. The objective of our study was to compare the direct effects of ethylenediaminetetraacetic acid (EDTA) and sodium citrate anticoagulants on platelet structure and PMP vesiculation using transmission electron microscopy to visualize the morphologic changes in platelets. Micrographs revealed that platelets in the EDTA-anticoagulated tube manifested with significant morphologic changes and induced PMP vesiculation. On the other hand, the sodium citrate-anticoagulated tube showed a normal platelet structure and minor modifications in some cases, with poor indication of PMP vesiculation. In conclusion, EDTA induced platelet activation and PMP vesiculation and represents a major source of artifacts during the pre-analysis steps of PMP vesiculation.

Introduction

Platelet microparticles (PMPs) are small fragments released from platelet cell membranes as a result of cell activation or apoptosis.[1] They contribute to a majority (approximately 70–90%) of the total circulating microparticles.[2,3] The diameter of PMPs ranges from 0.2 to 1 μm; they expose the antigens that represent the parent cell and negatively charged phospholipid phosphatidylserine (PS) as a result of remodeling of platelet plasma membrane. Nowadays, PMPs are well-known as carriers of bioactive molecules that play a role in physiologic and pathologic conditions such as blood coagulation, cell activation, inflammation, and cancer.[2,3] Scientific evidence reveals that PMPs have a high association with thrombi formation. For example, patients with deficiency in PMPs or Castaman's defect are at a very high risk of bleeding and tend to show an increased duration pertaining to the bleeding time, despite normal platelet count.[4] Moreover, in Scott's syndrome, in which platelets are unable to release microparticles, patients are more prone to bleeding diathesis.[5] In clinical practice, circulating PMPs that originate from activated human platelets are increased in various conditions such as prothrombotic disorders, cardiovascular diseases, inflammatory disorders, cancer, infectious diseases, and autoimmune diseases.[5,6] In these clinical conditions, the PMP count can serve as a useful tool to identify patients at risk of thrombotic or vascular disorders and evaluate treatment response.[7] Therefore, accurate measurement of PMPs is important.

The pre-analytical steps before PMP evaluation represent a potential source of variability and significant artifacts in PMPs preparation. One cause of this variability is the type of anticoagulant used during blood sample collection for PMP analysis. In the last decade, different types of anticoagulants were used for PMP preparation and isolation. It is imperative to consider how these anticoagulants may help reduce platelet activation during blood sample collection and preparation of plasma because platelet activation results in PMP formation and increased PMP count.[8] According to Connor et al.,[9] the concentrations of procoagulant phospholipid and Annexin V-positive PMPs in the sodium citrate-anticoagulated tube increased during the first 60 min of sample collection compared with their concentrations in the EDTA tube. They concluded that, when blood samples are not directly processed, PMP preparation should be performed on EDTA-anticoagulated samples. Another study supported the fact that EDTA is a better anticoagulant than sodium citrate in terms of PMPs analysis.[10] On the other hand, many scientists supported the idea that sodium citrate is more effective than EDTA for PMP analysis.[11–15] However, no study has compared the direct effect of

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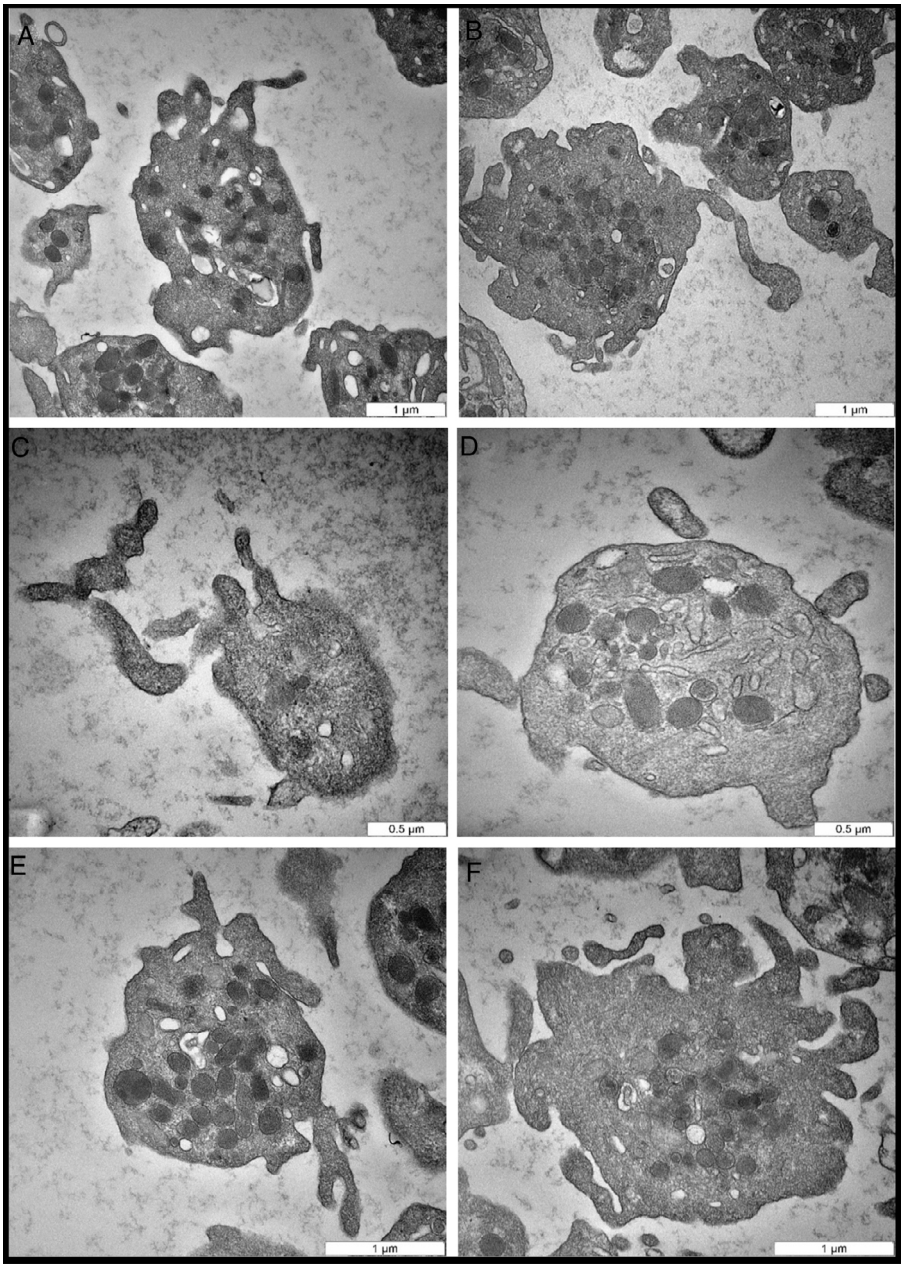

Fig. 1. Representative transmission electron microscopy micrographs of EDTA-induced changes. (A) Activated platelets with significant morphologic changes in the cell membrane, including elongation of projections. (B) Large activated platelet in the center shows potential morphologic changes in the alpha granule bodies, which are preparing to condense and fuse with each other. (C) Extended platelet projections and initiation of PMP vesiculation. (D) Intact activated platelet with cell membrane fusion and PMP formation. Note the dense bodies, and projection of the cell membrane. (E) The cell membrane projections, and condensation of alpha organelles. (F) Platelet in the advanced stage of activation, in which the cell membrane is damaged. PMPs, and other cell organelles, such as dense bodies, and alpha granules, are released.

EDTA and sodium citrate[16] and published data on related topics are few.[17,18] Therefore, there is currently no recommendation for the ideal anticoagulant for PMP analysis. The objective of our study was to compare the direct effects of EDTA and sodium citrate on normal human platelets, including PMP vesiculation.

Methods

Blood sample collection

After obtaining ethical approval and informed consent, blood samples were drawn from eight healthy volunteers not taking any medications and with normal hematologic parameters, such as platelet count, hemoglobin, white blood cell count, and differential count. Blood samples were drawn simultaneously from all subjects. Samples were drawn from the antecubital vein without using a tourniquet and while the subject was lying in a supine position. A butterfly device (blood collection set, 21-G) was used to collect the blood samples. The initial 3 ml of extracted blood was discarded to avoid artifacts. Blood samples were collected in 3.2% (0.109 M) citrated plastic tube (Vacuette, 3.5 ml; Greiner Bio-one, Frickenhausen, Germany) and EDTA tube (Venosafe, Terumo, Somerset, NJ, USA).

Sample processing

All sodium citrate- and EDTA-anticoagulated blood samples were processed by the same operator, in the same way. Samples

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