□ Case Report □

Prevention and Dissociation of the Platelet Aggregation in a Patient with EDTA-dependent Pseudothrombocytopenia by Supplementation of Kanamycin : A Case Report

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Pseudothrombocytopenia is usually associated with anticoagulant ethylene diaminetetraacetic acid (EDTA). The platelet clumping that occurs in EDTA-dependent pseudothrombocytopenia (EDPT) can sometimes be prevented by the use of other anticoagulants such as heparin or sodium citrate. As an alternative, we used kanamycin before or after the withdrawal of EDTA-anticoagulated blood in a 6-year-old boy with EDPT. Kanamycin used supplementarily during the differentiation of EDPT effectively prevented platelet clumping. **(Korean J Pediatr 2005;48:675-677)**

Key Words : Pseudothrombocytopenia, EDTA, Kanamycin

Introduction

Pseudothrombocytopenia is a spuriously low platelet count due to platelet clumping, which occurs in anticoagulated blood at room temperature. As automatic analyzers cannot count platelet clumps, they yield falsely low platelet counts notwithstanding normal platelet counts. Although sodium citrate, oxalate, and heparin have also been implicated, ethylene diaminetetraacetic acid (EDTA)-dependent platelet aggregation is the most frequent cause of pseudothrombocytopenia[1].

The incidence was estimated to be up 0.1% of all complete blood counts[2, 3]. Pseudothrombocytopenia may lead to an erroneous diagnosis, unnecessary examination, and sometimes inappropriate treatment. Recently, it was documented that the platelet clumping by EDTA could be prevented and dissociated by the supplementation of an aminoglycoside[4]. We present a case with EDTA-dependent pseudothrombocytopenia (EDPT) in a 6 year-old boy, which was corrected by the supplementation of kanamycin. To our knowledge, this is the first description of the phenomenon in children.

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Case Report

A 6 year-old Korean boy was born without any complications. His family history was unremarkable and he grew up without any specific health problems. He visited the Pediatric Department of Gil Medical Center for a high fever and cough. A physical examination revealed normal findings except for mild pharyngeal injection. There was no bruising or hemorrhage.

His platelet count on the first day admission was 72,000/mm^3. Platelet count in EDTA-anticoagulated blood on next day was 21,000/mm^3 with lots of platelet aggregations seen in blood smear. We compared the platelet counts between EDTA-anticoagulated, heparin-anticoagulated, and sodium citrate-anticoagulated blood. The platelet counts were 27,000/mm^3, 23,000/mm^3, and 479,000/mm^3, respectively. Then, we checked the platelet counts before or after supplementation of kanamycin to EDTA-anticoagulated blood. Twenty mg of kanamycin was added to EDTA tubes before or after blood sampling. The platelet counts were 661,000/mm^3 and 611,000/mm^3, respectively. However, the platelet count of the EDTA-anticoagulated blood without kanamycin supplementation was 27,000/mm^3. In addition, we could not observe any more platelet clumps in the films of kanamycin supplemented blood (Fig. 1).

Platelet autoantibodies were negative according to a

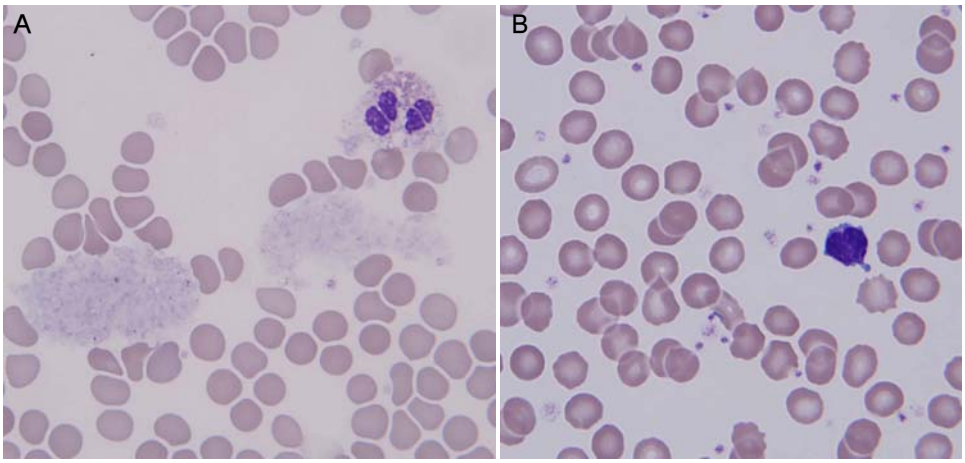

Fig. 1. Platelet clumps are noted in EDTA-anticoagulated blood **(A)**, and disappeared after the supplemental use of kanamycin **(B)** (Wright and Giemsa, ×400).

mixed passive hemoagglutination test. There were no other hematologic abnormalities, including prothrombin time, activated prothromboplastin time, fibrinogen level, and fibrin degeneration product level. Thereafter, his platelet count were serially followed. The platelet count of EDTA-anticoagulated blood without kanamycin still remained around 20,000/mm^3 in for 6 months without another hemorrhagic tendency.

Discussion

Pseudothrombocytopenia is an immunologically mediated phenomenon caused by the anticoagulant dependent cold antiplatelet autoantibodies in blood that cause platelet clumping. As the automatic instruments for the analysis and counting of blood cells cannot count a platelet clump as an individual platelet, spurious thrombocytopenia can occur. To date it is not known whether any specific diseases, including autoimmune diseases and drugs, are correlated with pseudothrombocytopenia. It has not been associated with hemorrhagic diathesis or platelet dysfunction. Although the incidence is as low as 0.1%, when considering the frequency of a complete blood cell counts test, pseudothrombocytopenia can be encountered easily. Sometimes spurious thrombocytopenia results in an unnecessary examination and treatment. Therefore, it is mandatory to exclude the conditions associated with pseudo from a variety of conditions causing thrombocytopenia.

Platelet clumping occurs most often in blood samples anticoagulated with EDTA, although sodium citrate, oxalate, and heparin have also been implicated[1]. EDTA-dependent platelet clumping is mediated by the binding of antiplatelet autoantibodies to platelet surface glycoproteins that are modified by the combined action of an anticoagulant and low temperature[5, 6]. These autoantibodies belong to the IgG, IgM or IgA class of immunoglobulins[7, 8]. The downregulation of GPIIb/IIIa in EDTA-blood of EDPT subjects and platelets from patients with Glanzmann's disease do not react with EDTA-dependent antibodies suggest the GPIIb/IIIa complex might be the binding site for the autoantibodies[8].

For the differentiation of pseudothrombocytopenia from true thrombocytopenia, direct visualization of the platelet clump under the microscope might be confirmatory. However, it has been recommended, as a matter of convenience, to collect and examine EDTA-anticoagulated blood at 37℃ or use the another anticoagulants (sodium citrate, oxalate and heparin) instead of EDTA. Aminoglycoside, although the precise mechanism is still unknown, can be used before blood sampling or after the withdrawal of EDTA-anticoagulated blood to prevent the platelet clumping that occurs in EDPT[4].

In our case, we examined whether platelet clumping could be prevented by the use of sodium citrate or heparin. The blood anticoagulated with heparin still showed spurious thrombocytopenia. But, sodium citrate could prevent the platelet clumping and showed normal platelet count. In addition, we examined whether an aminoglycoside could prevent platelet clumping by adding kanamycin before or after EDTA anticoagulated blood withdrawal. The blood treated with kanamycin had no platelet clump and showed normal platelet count.

In summary, we believe that this might be the first reported case of a child with EDPT corrected by the addition of aminoglycoside. The importance of this case is not just that it is the first described, but also that EDPT in children should be differentiated from true thrombocytopenia and this could easily be done by the use of an aminoglycoside.

한글 요약

EDTA 의존성 가성혈소판감소증 환아에서 가나마이신 보충에 의해 혈소판 응집을 예방할 수 있었던 1례

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가성혈소판감소증은 항응고제인 ethylenediaminetetraacetic acid (EDTA)를 사용 시 주로 발생한다. 이 때 관찰되는 혈소판 응고는 헤파린이나 구연산나트륨 같은 항응고제를 EDTA 대신 사용하면 일어나지 않기도 하여 가성혈소판감소증과 진성혈소판감소증을 감별할 수 있다. 저자들은 이러한 감별을 위하여 6세 된 EDTA 의존성 가성혈소판감소증 남아에서 아미노글리코사이드 계열의 약물인 가나마이신을 채혈 전에 EDTA 용기에 미리 넣거나, EDTA 용기에 이미 채혈된 혈액에 첨가한 후 각각 혈소판수와 혈소판 응고 양상을 조사하여 보았다. 그 결과 가나마이신 채혈 전 혹은 후에 처리한 경우 모두 혈소판응고 없이 정상적인 혈소판수를 나타내어 소아에서도 임상적으로 쉽게 검사할 수 있는 방법으로 생각되어 보고하는 바이다.

References

1) Bizzaro N. EDTA-dependent pseudothrombocytopenia : a clinical and epidemiological study of 112 cases, with 10-year follow-up. Am J Hematol 1995;50:103-9.
2) Onder O, Weinstein A, Hoyer LW. Pseudothrombocytopenia caused by platelet agglutinins that are reactive in blood anticoagulated with chelating agents. Blood 1980;56:177-82.
3) Payne BA, Pierre RV. Pseudothrombocytopenia : a laboratory artifact with potentially serious consequences. Mayo Clin Proc 1984;59:123-5.
4) Sakurai S, Shiojima I, Tanigawa T, Nakahara K. Aminoglycosides prevent and dissociate the aggregation of platelet in patients with EDTA-dependent pseudothrombocytopenia. Br J Haematol 1997;99:817-23.
5) Van Vliet HH, Kappers-Klunne MC, Abels J. Pseudothrombocytopenia : a cold autoantibodies against platelet glycoprotein GP IIb. Br J Haematol 1986;62:501-11.
6) Kunicki TJ, Pidard D, Rosa JP, Nurden AT. The formation of Ca^{++} dependent complexes of platelet membrane glycoproteins IIb and IIIa in solution as determined by crossed immunoelectrophoresis. Blood 1981;58:268-78.
7) Pegels JG, Bruynes EC, Engelfriet CP, von dem Borne AE. Pseudothrombocytopenia : an immunologic study on platelet antibodies dependent on ethylene diamine tetra-acetate. Blood 1982;59:157-61.
8) Casonato A, Bertomoro A, Pontara E, Dannhauser D, Lazzaro AR, Girolami A. EDTA dependent pseudothrombocytopenia caused by antibodies against the cytoadhesive receptor of platelet GPIIb/IIIa. J Clin Pathol 1994;47:625-30.