Autologous Platelet Collection and Storage to Support Thrombocytopenia in a Leukemia Patient with Platelet Alloimmunization Undergoing Chemotherapy

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ABSTRACT

Platelet alloimmunization occurs frequently in multitransfused patients. To prevent the posttransfusion complications donor white blood cells (WBCs) must be removed from the platelet concentrates (PC). But sometimes the centrifugation and filtration of WBC is not enough and in such conditions HLA-matched PC are recommended. Cryopreservation of autologous PC offers a potential solution to this problem. We investigated the feasibility of supporting the aplastic period after chemotherapy in a 54-year-old leukemia patient with autologous platelets collected by apheresis and cryopreserved. Our case report demonstrates that autologous apheresis platelet transfusion might be a feasible approach to high risk patients with bleeding tendencies.

Key Words: Blood transfusion autologous, Platelet, İmmunization, Transfusion, Allogeneic.

ÖZET

Kemoterapi Sonrası Trombosit Alloimmünizasyonu Gelişen Lösemili Bir Hastada Otolog Trombosit Aferezi ve Saklanması

Trombosit alloimmünizasyonu çoklu transfüzyon yapılan bireylerde karşılaşılan önemli bir problemdir. Transfüzyon sonrası gelişecek komplikasyonları önlemek için transfüze edilecek üründeki lökositlerin uzaklaştırılması gerekmektedir. Ancak bazen bu işlem için uygulanan santrifügasyon ve filtrasyon yeterli olmamaktadır, bu durumda HLA uyumlu trombosit transfüzyonu önerilmektedir. Bir başka çözüm de kriyopreserve edilen otolog trombositlerin transfüzyonudur. Bu olgu sunumunda, indüksiyon tedavisi sırasında trombosit refrakterliği gelişen akut lösemili bir olguda tromboferez sonrası kriyopreserve edilen otolog trombosit transfüzyonunun yararı incelendi.

Anahtar Kelimeler: Kan transfüzyonu, Trombosit, İmmünizasyon, Transfüzyon, Allogeneik.

Turk J Haematol 2003;20(4): 233-236

Received: *16.03.2003* ***Accepted:*** *26.09.2003*

INTRODUCTION

Platelet alloimmunization occurs frequently in multitransfused patients with hematological malignancies. Platelet alloantibodies are directed against allotypic determinants that are genetically expressed on platelet surfaces and reflected by structural differences of glycoproteins. This problem affects patients' hemostatic status at the further treatment period. Measures such as single donor platelets, strict blood group recognition and leukocyte filters are not always effective, and in such conditions HLA matched platelet concentrates (PC) are recommended[1]. Cryopreservation of autologous PC offers a potential solution to this problem. Moreover, cryopreservation could allow the use of HLA matched PC for refractory patients and platelet auto-transfusion for patients undergoing intensive chemotherapy. We investigated the feasibility of supporting the aplasia period with autologous platelets collected by apheresis and cryopreserved.

A CASE REPORT

A 54-year-old woman was admitted to the hospital because of fatigue, echymosis, anorexia, weight loss and fever. At the admission her CBC was as follows; Hb: 9.6 g/dL, Htc: 27.2%, Plt: 23 x 10^9/L, white blood cells (WBC): 15.8 x 10^9/L. She was diagnosed as de novo acute myeloblastic leukemia (AML), M_1. Remission induction therapy consisting cytarabine (Ara-C) 100 mg/m^2 IV continuous infusion day 1-7, and idarubicin (IDA) 10 mg/m^2 IV short infusion day 1-3 and rhG-CSF for 20 days was administered. Myeloablation induced pancytopenia was observed till the 26th day of chemotherapy. During this period the patient was refractory to random donor platelets and single donor apheresis platelet concentrates. Until the end of therapy 16 units of single donor platelets were transfused with limited success. Her hematopoietic recovery was on the 26th day of chemotherapy. Bone marrow examination revealed that the patient was in 1st complete remission and she was discharged in a good performance status. At her second admission for the first consolidation therapy her CBC parameters were as follows; hemoglobin: 11.5 g/dL, hematocrit: 32.8%, platelet: 266 x 10^9/L and WBC: 4 x 10^9/L. There was no blast on her blood smear. As her remission induction therapy was complicated by the platelet refractoriness, autologous plateletpheresis was planned before the consolidation therapy. Plateletpheresis was performed on a COBE Spectra (Lakewood, Colorado) continuous flow cell separator. The plateletpheresis product containing 3.3 x 10^{11} platelets was cryopreserved with DMSO to a final concentration of 5% in a rate-controlled freezer to -120°C. For consolidation therapy ARA-C 500 mg/m^2 b.i.d. as 2 hours IV infusion for days 1-6 and IDA 10 mg/m^2 IV for days 1-3. On the 5th day of chemotherapy platelet count was 100 x 10^9/L and after than gradually dropped to 40 x 10^9/L on day 10. On the 11th day the platelet count was 17 x 10^9/L and a febril attack developed. Though 1 unit of single donor platelets were transfused and the fever was controlled, on the 11th day platelet count was still 23 x 10^9/L one hour after transfusion. The platelet count was 4 x 10^9/L on the 13th day and after thawing and transfusing her autologous platelets her platelet count rose to the level of 68 x 10^9/L, which has never been achieved by the infusion of allogeneic and filtered single donor platelet concentrates.

DISCUSSION

Platelet refractoriness is one of the most important life threatening states during aplastic period. Fever, bleeding, splenomegaly and alloimmunization are the factors that cause refractoriness. In our case neither fever nor bleeding was present and though we could not show the alloantibodies against GPIIb/IIIa complex or HLA class I molecules we assumed that alloimmunization was the cause. We investigated the feasibility of supporting the aplastic period with autologous platelets.

Early attempts to store platelets were performed, as whole blood or red blood cells at 4°C. However, refrigerated storage was soon abandoned due to the finding that cold temperatures significantly and irreversibly affect platelets. The risk of the bacteremia associated with transfusion of room temperature stored PCs, has renewed the interest for development of platelet cold storage. In order to prevent cold induced platelet activation several physical and biochemical methods have been explored, such as, increased atmospheric pressures, temperature cycling, cytoskeletal stabilisers, antifreeze glycoproteins, signal transduction inhibitors. Several drugs, such as taxol as a microtubule stabiliser, cytochalasin as an inhibitor of actin filament assembly, Quin2 as a cytoplasmic calcium chelator have been used to prevent platelet changes induced by chilling and to maintain the discoid shape of platelets and physiological responses[2].

As for many cell types, freezing has long been considered an alternative for platelet preservation, with major advantage of extending the storage period from a few days to years. Several studies have proven the usefulness of frozen platelets in the prophylaxis of bleeding in different clinical settings (cardiopulmonary by-pass, onco-haematological patients undergoing high dose chemotherapy and/or haematopoietic progenitor cell transplantation). Some of the cryoprotectant agent for platelet freezing are, glycerol based solutions, hydroxyethylstarch, trephalose, propane-1,2-diol and most widely used agent dimethyl sulphoxide (DMSO)[2,3].

Platelet alloimmunization occurs frequently in multitransfused patients. Blood components contain variable amounts of donor WBCs. WBCs in blood transfusions can cause adverse effects in the recipient: alloimmunization against WBC (HLA) antigens, nonhemolytic febrile reactions after transfusion, refractoriness to platelet transfusions and transmission of certain infectious agents. To prevent these posttransfusion complications, donor WBCs must be removed from the PC before transfusion by centrifugation and/or filtration[3].

Platelet preparation and storage procedures may play a significant role in release of leukocyte and platelet derived bioactive substances. Previous studies have shown that preparation of the PCs by the platelet-rich plasma (PRP) method leads to a significant time dependent accumulation of extracellular histamine, interleukin (IL)-1, IL-1α, IL-6, IL-8 and tumour necrosis factor (TNF)-α, which eventually may be prevented or reduced by leukocyte filtration of PCs either at bedside or pre-storage[4,5].

Currently, owing to open system pooling techniques, it is acceptable to store pooled platelets only for four hours. Longer storage times are contraindicated owing to concerns over bacterial contamination while pooling and risk of subsequent bacterial growth during storage[6].

The best method for platelet cryopreservation still remains an object of debate. Some authors observed that platelets frozen and thawed in the presence of glycerol had good in vitro and in vivo properties, while some others concluded that such a treatment rendered platelets unsuitable for clinical use. In contrast majority of the authors who studied DMSO cryopreserved platelets described good in vitro and in vivo results. Though these reports from the literature seem to indicate that DMSO is a safer cryopreservative than glycerol, direct evidence of this superiority is still inadequate[1].

To further investigate this important field, Balduni studied additional functional characteristics of platelets cryopreserved with two well defined methods employing DMSO and glycerol-glucose, respectively. In this study both of the methods gave a good platelet yield after freezing, thawing and washing. The in vivo hemostatic role of PC cryopreserved with the two methods is difficult to asses on the basis of in vitro functional tests[1]. Since DMSO has well recognized adverse ef-

fects, a reduction of its concentration in platelet freezing or substitution by less toxic cryoprotectant regimens is desirable to allow direct infusion of frozen thawed platelets. In this regard, PCs frozen with a solution containing 20% polyvinyl-pyrolidone, 10% mannitol, 5% glycerol and a mixture of salts appear to function well when infused to thrombocytopenic rabbits without post-thaw washing of the cryoprotectants. It has been observed that platelets frozen with DMSO concentration of 2% combined with ThromboSol mixture (amiloride, sodium nitropurusside and adenosine) display in vitro properties similar to those cells cryopreserved with the standard 6% concentration of DMSO[7].

One way to support thrombocytopenic patients who are refractory to randomly selected platelets is, using HLA-matched platelets and crossmatch-compatible platelets. Data supporting the effectiveness of crossmatch-compatible platelets are limited[8].

CONCLUSION

The contaminating WBCs and the problem of alloimmunization could be overcome with cryopreservation of autologous platelets before myeloablative therapy. The cost effectiveness of this approach should be tested in randomized studies. In our case report we emphasized the problem of alloimmunization and demonstrated that autologous apheresis platelet transfusion might be a feasible approach in high risk patients, with bleeding tendencies.

Cryopreservation of autologous platelets has been successfully utilized in patients with problems related to alloimmunization and isoimmunization but the platelet yield is less than with fresh platelets and the technique is limited by the need for extra processing and availability of storage space. Improved understanding of platelet physiology has led to additional approaches to obtain a platelet substitute. Several investigators have been able to introduce platelet glycoproteins into liposomes for in vitro experiments. Also it is theorically possible that thrombo-erythrocytes could function as an autologuous, semi artificial platelet alternative[9,10].

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