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Automatic Isolation of Bone Marrow Mononuclesr Cells Using Fenwal CS-3000 Plus Blood Cell Separator

Shinji Kamei, Kenji Shinohara*, Toshiaki Yujiri*, Kazuhiko Shigemoto*, Eiichi Oeda* and Hiroki Yohena***

*Division of Hematology, Department of Medicine, Yamaguchi Prefecture Central Hospital, Hofu, Yamaguchi 747. Japan **Baxter Limited, Tokyo 102, Japan
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Abstract Bone marrow mononuclear cells were separated by continuous centrifugation with a Fenwal CS-3000 Plus Blood Cell Separator. This procedure has been performed by automatic processing of bone marrow aspirate in a closed sterile system, without the use of density gradient, and took approximately 1 hour for processing 500 ml of bone marrow aspirate. The recovery of the mononuclear cells was 17.8±10.0% of the total nucleated cells, with negligible red cell and granulocyte contamination, allowing the subsequent freezing to be carried out directly. Hematological recovery after autologous bone marrow transplantation was prompt with these cells, and took 14 days. This method of separation of mononuclear celle is suitable for use in ABO-incompatible allogeneic and in autologous bone marrow transplantation.

Key Words : Bone marrow transplantation, Mononuclear cell

Introduction

The separation of bone marrow mononuclear cells containing hematopoietic stem cells from bone marrow aspirate is necessary for red cell depletion in ABO-incompatible allogeneic bone marrow transplantation and for cryopreservation in autologous bone marrow transplantation. Several methods have been used for this purpose employing blood cell separators or blood cell processors. Some of these methods are; physical separation by centrifugation, using a blood cell separator such as Hemonetics Blood Cell Separator[1)-3)], and separation by density gradient centrifugation using an IBM(currently Cobe)-299I Blood Cell Processor[4)-7)] or Hemonetics Model 30 Blood Cell Separator[8)] and Ficoll-Hypaque or Percoll for density gradient. However, the former method has the disadvantage of heavy red cell contamination, while the latter two methods necessitated a subsequent step, i.e., removal of the agents used for the density gradient.

A new model, Fenwal CS-3000 Plus Blood Cell Separator, has recently been manufactured; it is equipped with an automatic device which separates mononuclear cells from bone marrow aspirate by physical separation with continuous centrifugation, without employing density gradients or sedimenting agents[9)-11)]. To date, there have been few reports of experience with this apparatus[9)-11)]. Accordingly, we used this apparatus; separation method was simple, there was excellent recovery of mononuclear cells which contained hematopoietic stem cellls, and the hematological recovery after autologous bone marrow transplantation with these cells was prompt, and took 14 days.

Patients

Five patients (4 with malignant lymphoma and 1 with acute lymphoblastic leukemia)

Table 1. Yields of bone marrow cells.
a. Granulocyte colony-stimulating factor(G-CSF), at 2.5μg/kg of body weight, was administered for 7 days prior to bone marrow cell harvest.

Case G-CSF	1	2	3_a (+)	4_a (+)	5_a (+)	Mean±SD
Nucleated Cells($\times 10^8$/kg)	3.3	4.6	7.1	10.1	7.2	6.5± 2.6
Monoculear Cells($\times 10^8$/kg)	0.4	1.1	2.3	1.1	0.7	1.1± 0.7
Recovery(%)	12.1	23.9	32.4	10.9	9.7	17.8±10.0

were the candidates for harvesting bone marrow cells for autologous bone marrow transplantation (Table 1). Three of the patients received granulocyte colony-stimulating factor (G-CSF), 2.5 μg/kg of body weight/day, for 7 days prior to harvesting of bone marrow cells. Approximately 1000 ml of bone marrow aspirate, which was expected to contain at least $3x10^8$ nucleated cells per kg of body weight, was aspirated from the bilateral iliac bones and was collected in an equal volume of medium containing 50u/ml of heparin, while patients were under general anesthesia. After autologous bone marrow transplantation, all the patients received G-CSF, 5μg/kg of body weight, to accelerate hematological recovery.

Methods

The Fenwal CS-3000 Plus Blood Cell Separator is a continuous-flow centrifugal device (Baxter Healthcare Corporation, Deerfield, IL, USA). Plus denotes the newly added automatic program to the former CS-3000 model for the separation of mononuclear cells from bone marrow aspirate based on the established program for lymphocytapheresis[11].

A diagram of this apparatus is shown in Figure 1. The automatic processing program outlined below requires minimal operator interevention, and is processed automatically in the Auto Run mode[11]. A closed circulation of disposable apheresis kit was primed with saline containing 1.25% albumin from the inlet of normal saline. The bone marrow cell suspension (1000ml) was pumped into the circulation from the inlet of starting blood at 25 ml/min mixing with ACD solution, and then centrifuged at 1400 rpm for 40 min. As

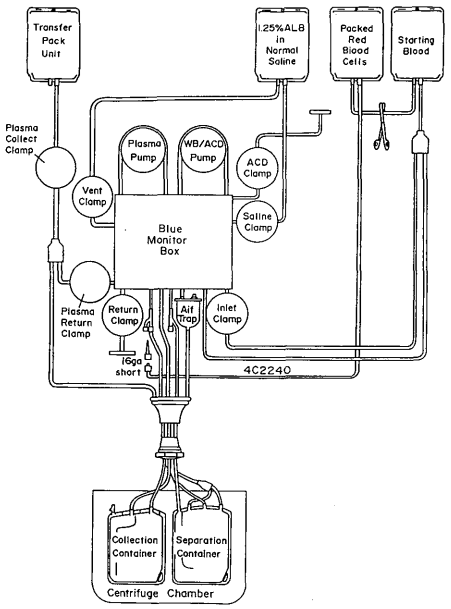

Fig 1. Diagram of the Fenwal GS-3000 Plus Blood Cell Separator. Referred from the operating manual of GS-3000 Plus procedure for processing bone marrow. See text for operating details.

shown in Figure 2, mononuclear cell fraction was separated into a 200-ml collection container. The separated bone marrow mononuclear cells were mixed with an equal amount of medium containing 20% serum and dimethyl sulfoxide. The cells were then collected in a freezing bag and program frozen to -80 ° C, then they were subsequently stored in liquid nitrogen. At the time of autologous bone marrow transplantation, the bone marrow cells were rapidly thawed at 37 ° C in an incubator and administered intravenously to

the patients.

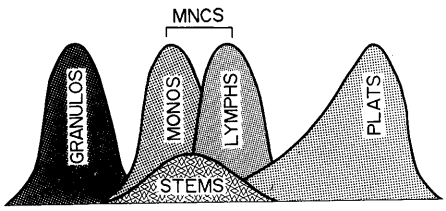

Fig 2. Scheme showing density distribution of bone marrow blood cells.The mononuclear cells(MNCS) fraction was collected.
Plats:platelets
Lymphs:lymphocytes
Monos:monocytes
Stems:stem cells
Granulos:granulocytes

Results

The yields of the bone marrow cells from the individual patients are shown in Table 1. The average number of bone marrow cells aspirated was $6.5\pm2.6\times10^8$/kg of body weight, and the number of mononuclear cells separated was $1.1\pm0.7\times10^8$/kg of body weight. The recovery rate of mononuclear cells was $17.8\pm10.0\%$ of the total nucleated cells. Contaminations of red cells and granulocytes were approximately of 1% and 10%, respectively, of those initially present in the bone marrow aspirate. The original volume, 1000 ml, of the bone marrow aspirate was reduced to 400 ml after separation of the mononuclear cells. At the time of writing, four patients have already received autologous bone marrow transplantation. Days elapsed until hematological recovery, as assessed by granulocyte recovery of more than $500/\mu l$ and platelet recovery of more than $2.0\times10^4/\mu l$, were $13.7+5.0$ as shown in Table 2. Details of the clinical courses of these patients will be presented elsewhere.

Table 2. Days required for recovery of hematopoiesis after autologous bone marrow transplantation.
NE:not examined. This patient died of cerebral hemorrhage before hematological recovery on day 15.

Case	Granulocyte	Platelet
1	NE	NE
2	19	19
3	9	9
4	13	13
Mean±SD	13.7±5.0	13.7±5.0

Discussion

This method of separating bone marrow mononuclear cells using the Fenwal CS-3000 Plus Blood Cell Separator has following advantages over previous methods. The separation method is simpler, only one separation process being necessary. The step in which removal of the agent used for density gradients, such as Ficoll-Hypaque, is not necessary; the closed separation system yields an aseptic separated product; red cell and granulocyte contamination is negligible; and the recovery of mononuclear cells is excellent. A munual procedure for stem cell concentration in test tubes was performed using Ficoll-Hypaque as a density gradient[12]. This technique gave a product similiar to that obtained with blood cell separators using density gradients; however, it is extremely time- and labor-consuming and has a higher risk of bacterial contamination during processing. Separation of mononuclear cells using a blood cell separator such as Hemonetics, which employs a discontinuous-flow system, yields a buffy coat in which approximately 80% of mononuclear celle and 60-80% of granulocyte macrophage colony-forming hematopoietic stem cells are recovered; however, red cells contamination is heavy at 6%-12%[1)-3)]. Separation using the IBM (currently Cobel)-2991 Blood Cell Processor,or Hemonetics Model 30 Blood Cell Separator and Ficoll-Hypaque or Percoll as density gradients resulted in an excellent recovery of mononuclear cells and with little contamination by red cells and granulocytes, however, it requires the subsequent step of removing the agents use for density gradients[4)-8)].

The present method of separation is

similiar to the method using the IBM (currently Cobe)-2997 Blood Cell Separator with regard to the procedure for separation by continuous flow centrifugation. With that method, the mononuclear cell concentrate was isolated in 15% of the original bone marrow cell volume, and contained 23% of the nucleated cells; contamination by granulocytes, red cells and platelets was 7.2%, 1.5% and 41%, respectively of cells initially present in the bone marrow cell suspension[12]. Our results were comparable to those data, as well as to those previously reported for CS-3000[9)-11)]. We did not perform in vitro assay of hematopoietic stem cells, and thus the recovery rate of stem cells is not known: however, hematological recovery including hemoglobin, leukocyte and platelet after autologous bone marrow transplantation was prompt and suggested sufficient recovery of the hematopoietic stem cells.

G-CSF and granulocyte macrophage colony-stimulating factor act not only on granulocyte and macrophage committed stem cells but also on multipotent stem cells[14]. Administration of G-CSF might be useful for enrichment of hematopoietic stem cells in the peripheral blood and bone marrow before bone marrow cell harvest, and is also useful for accelerating hematopoietic recovery after bone marrow transplantation[15]. In our experience, the number of nucleated cells was increased in the patients who had received G-CSF before bone marrow cells harvest, however, an increase in the number of mononuclear cells which include hematopoietic stem cells was not confirmed. The effect of G-CSF administration after autologous bone marrow transplantation was evident in the faster recovery of hematopoiesis compared with the previous data in which it took approximately 3-4 weeks without the use of G-CSF[15].

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