Efficacy of the Mobilization of Peripheral Blood Stem Cells by Granulocyte Colony-Stimulating Factor in Pediatric Donors

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Abstract

The advantages/disadvantages of the use of peripheral blood stem cells (PBSCs) for allogeneic transplantation still need to be clarified, particularly in children. We compared the kinetics, efficacy, and safety of PBSC mobilization by granulocyte colony-stimulating factor (G-CSF) and collection by apheresis between healthy pediatric and adult donors. A total of 19 pediatric (median age, 6 years) and 25 adult healthy donors (median age, 37 years) were given 10 μg/kg/day of G-CSF for 5 consecutive days for PBSC mobilization, which were harvested by apheresis on days 5 and/or 6. All of the donors tolerated the whole procedures. Serum trough levels of G-CSF determined by ELISA were significantly lower in the 16 pediatric donors evaluated than in adults (P < 0.05). Although the WBC counts on days 4 and 5 were significantly higher in adults than in children (P = 0.006 and 0.004, respectively), the numbers of circulating CD34⁺ cells/unit of blood were identical. The number of blood CD34⁺ cells collected per unit of blood processed was identical in both donor populations. We propose that PBSCs could be effectively mobilized and collected in small children so that they could be donors for adult patients.

Introduction

Allogeneic PBSCCT is increasingly used in place of bone marrow transplantation (1). Its benefits include faster hematopoietic recovery and a lower complication rate in the early stage of transplantation (1). Its benefits include faster hematopoietic recovery.

Materials and Methods

Subjects and Mobilization Protocol. A total of 19 healthy children (9 males and 10 females) who were the related donors for allogeneic PBSCST was enrolled into this study. The ranges of their ages were 2–5 years in 6, 6–10 years in 4, and 11–16 years in 9 (median age, 10 years). PBSC mobilization was performed by the administration of 10 μg/kg/day of G-CSF (filgrastim from Kirin Brewery Co., lenograstim from Chugai Pharmaceutical Co., or nartograstim from Kyowa-Hakko Kogyo Co., Tokyo, Japan) for 5 consecutive days. Principally, G-CSF was injected s.c. at 9 a.m. each day. Blood samples for daily analysis of complete blood count, blood chemistry, colony assay, CD34⁺ cell assay, and the serum concentration of G-CSF were drawn immediately before the injection of G-CSF. Data from 25 adult donors (median age, 37 years; 18–49 years: 17 males and 8 females) who were treated with the same mobilization protocol were also analyzed. All of the adult donors received prophylactic aspirin (600–900 mg/day) during G-CSF treatment. A consent form was obtained from a guardian of the pediatric donors or directly from the adult donors. This trial was approved by the individual Institutional Review Boards.

PBSC Collection Procedure. On days 5 and/or 6 of the mobilization protocol, collection of PBSCs was begun at 11 a.m. using a Fenwal CS3000 Plus (Baxter Healthcare Co, Irvine, CA) with a processing volume of 300 ml/kg (maximum, 10 liters) per session. For small donors weighing <20 kg, a small volume separation chamber holder was used, and WBC-depleted, irradiated, washed RBCs were also used for priming the extracorporeal line before blood processing was begun, as reported previously (13). In four small donors, a total of 200 ml of previously collected and stored autologous RBCs was used for priming. Principally, blood was drawn from a catheter inserted into a radial artery and given back through an antecubital vein. In the case of small donors who used autologous RBCs, RBCs left in the apheresis kit were collected for the next procedure. To avoid hypocalcemia due to citrate intoxication, calcium gluconate was administered continuously in a ratio of 1 ml per 200 ml of blood processed. After small, the hematocrit level and percentage of oxygen saturation in the arterial blood were continuously monitored with a Crit-Line (IN-LINE Diagnostic Corp., UT) in pediatric donors (14).

In the case of adult donors, PBSCs were universally harvested with a standard CS3000 Plus using the combination of a granulocyte chamber and small volume collection chamber, as reported previously (15).

Hematopoietic Progenitor Assay. The details of the hematopoietic progenitor assays have been reported previously (16). Briefly, cells were incubated in methylcellulose medium supplemented with 20% fetal bovine serum (Life Technologies, Inc., Grand Island, NY), 450 μg/ml of human transferrin (Sigma Chemical Co., St. Louis, MO), 2 units/ml of human recombinant erythropoietin (Kirin Brewery Co.), 1% crystalized BSA (Calbiochem 12567; Hoechst Japan, Tokyo, Japan), interleukin 3 (20 ng/ml; Kirin), stem cell factor (20 ng/ml; Kirin), and G-CSF (20 ng/ml; filgrastim, Kirin). Cells were placed into 24-well culture plates (Corning, New York, NY) in quadruplicate and cultured for 14 days. The numbers of colonies were determined by morphological analysis.

Received 3/8/99; accepted 6/1/99.

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1 This work was supported by a Grant-in-aid for Scientific Research (C) (2) from the Ministry of Education, Science, Sports and Culture and by a Grant-in-aid for the Second-term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health and Welfare.

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3 The abbreviations used are: PBSC, peripheral blood stem cell transplantation; G-CSF, granulocyte colony-stimulating factor; CFU-GM, colony forming units for granulocyte/macrophage; MNC, mononuclear cell.
incubated in an ESPEC N 2 -O 2 -CO 2 BNP-110 incubator (Tabai ESPEC Co., Osaka, Japan), which maintained a humid atmosphere of 5% carbon dioxide, 5% oxygen, and 90% nitrogen. After 14 days of incubation, the number of CFU-GM was counted using an inverted microscope.

Flow Cytometry Analysis of CD34+ Cells. Assay of CD34+ cells was performed exclusively by Ohtsuka Assay Institute (Tokyo, Japan) as a central laboratory. Sample cells were shipped by air cargo and assayed within 24 h. Cells that expressed the surface CD34 antigen were identified by flow cytometry analysis. Briefly, 100 µl of cell suspension were added to a test tube (Falcon 2052; Becton Dickinson, Lincoln Park, NJ) containing isotype control (phycoerythrin-mouse IgG1) and phycoerythrin-conjugated CD34 monoclonal antibody (Anti-HPCA2 antibody; Becton Dickinson) at a concentration of 1 µg of antibody/10⁶ cells. Samples were analyzed with a FACSscan flow cytometer (Becton Dickinson). After the function was verified, samples were drawn into the flow cytometer using forward side scatter and side scatter, as gating parameters, along with debris subtraction techniques to determine the characteristics of the cells. A total of 20,000 events were counted to identify the mononuclear cell fraction. The flow cytometric data were analyzed using a gated analysis via a set of SSC-FL parameters for CD34+ cells to calculate the percentage of positive cells. When the sample was substantially contaminated with RBCs, they were lysed with a solution consisting of 0.826% (w/v) NH₄Cl, 0.1% KHCO₃, and 0.004% EDTA-4Na.

Assay of G-CSF Concentration. An ELISA kit for human G-CSF was purchased from Amersham Life Science (Biotrek ELISA System, Buckinghamshire, United Kingdom). The manufacturer guaranteed the specificity of the assay by demonstrating that it failed to detect other known cytokines. The serum G-CSF level was measured by strictly adhering to the manufacturer’s instructions. In this assay system, the limit of sensitivity for the assay kit was 1.1 pg/ml for G-CSF.

Statistics. The numbers of MNCs were indirectly calculated from the total number of WBCs and percentages of MNCs (monocytes and lymphocytes) each day of G-CSF treatment. The circulating number of CD34+ cells was calculated from the number of MNCs and the percentage of CD34+ cells in the MNC fraction. The number of CFU-GM was determined by calculation using the number of colonies per unit number of MNC. The Mann Whitney U test and/or unpaired t test combined with the F test were used to analyze differences in the effect of G-CSF administration. Data were analyzed using the StatView program (version 4.5; Abacus Concepts, Inc., Berkeley, CA) for a Macintosh computer.

Results

Mobilization. During G-CSF treatment for mobilization, no detectable adverse symptoms or complaints were documented in younger pediatric donors (n = 9, <10 years), whereas 5 of the 10 older pediatric donors (>10 years) complained of mild headache and general fatigue and received 10 mg/kg of acetaminophen when required. In contrast, all of the adult donors (n = 25) had various complaints including severe lumbar pain, headache, systemic bone pain, and general fatigue, despite the prophylactic use of aspirin. Nevertheless, none of the donors failed to complete the mobilization and harvesting protocols.

The number of WBCs increased from day 2 of G-CSF treatment (24 h after the first injection) and reached a peak value on day 3 or 4 in all of the donors. The peak values of WBCs on days 4 and 5 were significantly lower in pediatric donors than in adult donors (Fig. 1), whereas there were no differences in MNCs (Fig. 2) or the percentage of CD34+ cells (children, 0.93 versus adults, 1.04) between the two
groups. As a result, the absolute numbers of circulating CD34+ cells per unit of blood were identical in both groups. Although the levels of hemoglobin and platelet counts did not significantly change during the G-CSF treatment, a marked decrease in the platelet level (<50 × 10^9/liter) developed in two adult donors after plural courses of aphereses (>40 liters of blood processed), in whom platelet-rich plasma was required to be given back after the final step of cell preparation. None of the donors developed a tendency to bleed during or after the treatment. All of the blood components recovered to baseline levels in all of the donors within 2 weeks after the aphereses.

G-CSF pharmacokinetics could be evaluated in 16 donors from each group and showed a wide interdonor variation in the serum trough level of G-CSF, which was significantly lower in pediatric than in adult donors on days 3 and 4 (Fig. 3). The trough G-CSF levels became maximal on the second day of treatment in most of the donors, with a subsequent decline upon continuing G-CSF administration.

**Apheresis Procedure.** Three adult patients complained of mild nausea and palpitation during apheresis, which quickly responded to the rapid additional injection of 5 ml of calcium gluconate. Consequently, none of the donors failed to complete apheresis. Crit-Line monitoring in selected donors disclosed no significant change in the dynamics of circulation. Changes in the values of blood components before and after apheresis were not clinically significant in any of the donors, although several cases required an add-back of platelet-rich plasma, as described above. Median numbers of MNC, CD34+ cells, and CFU-GM harvested from pediatric donors were, respectively, 360 × 10^6, 305 × 10^6, and 660 × 10^5; a statistically significant difference was found only in MNCs, when compared with those in adults (787 × 10^6, 463 × 10^6, and 747 × 10^5, respectively). As a result, the numbers of cells harvested per processed volume (liter) of blood or per kg of body weight were equivalent between adult and pediatric donors (Table 1).

**Discussion**

Although allogeneic PB SCT is presently being used more often in place of bone marrow transplantation, the safety of G-CSF administration in healthy normal donors has not yet been established. Possible adverse effects of G-CSF or PBSC collection by apheresis should be evaluated against the existing risks of multiple marrow aspirations under general anesthesia. Consequently, in adult donors, G-CSF administration is thought to be less invasive than marrow aspiration, and several advantages have been noted for both donors and recipients (17). Although a consensus has been made to not exclude pediatric donors from this procedure, there is no established protocol for the mobilization of PBSCs by G-CSF in pediatric donors (18). The required numbers of PBSCs will be primarily determined by the recipient’s body size, and in this regard, an improved mobilization and harvesting protocol should be established for children who will donate grafts for an older child or adult patient.

In this study, we noted for the first time that the serum kinetics of G-CSF differ between adult and pediatric donors undergoing PBSC mobilization. The trough level of G-CSF tended to become lower in almost all of the donors as mobilization continued. Watts et al. (19) reported that G-CSF concentrations on day 6 were one-third of those on day 1 in adult donors. Additionally, there have been reports that G-CSF clearance increases with an increase in the number of absolute neutrophil counts in mobilization settings (3, 20). Accordingly, Faulkner et al. (20) suggested that an absolute neutrophil counts-adjusted G-CSF dosing schedule may improve a PBSC mobilization protocol for patients with cancer (20). In this study with normal healthy donors, we showed that the decrease in the G-CSF concentration was more prominent in pediatric donors compared with adult donors, despite their lower number of absolute neutrophil counts.

Our pediatric donors also had a lower level of G-CSF than adult donors at any point in the evaluation (not statistically significant), which might be related to the lower frequency of G-CSF toxicity observed in our pediatric donors. Although careful evaluation is still required with a larger number of patients within each age range, it appears that G-CSF clearance may change during treatment in different degrees between children and adults. It has been reported that a dose-response effect exists in the use of G-CSF for mobilization. Taken together, these data may indicate that a pharmacokinetically adjusted higher dose of G-CSF may be indicated for effective mobilization from pediatric donors.

The present results suggest that G-CSF mobilization and harvesting of PBSCs are safe and effective in pediatric donors, as well as in adult donors. Although a long-term follow-up is still required to confirm the safety of G-CSF treatment, children appear to tolerate this drug well while demonstrating the same mobilization effect as adult donors.

**References**


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