Granulocyte Colony-Stimulating Factor Increases the Platelet Volume in Peripheral Stem Cell Apheresis Donors

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Key Words

Granulocyte colony-stimulating factor • Mean platelet volume • Allogeneic peripheral blood stem cell transplantation donor

Abstract

We investigated the short-term influence of granulocyte colony-stimulating factor (G-CSF) administration on platelet counts and platelet indices in 12 donors (8 males and 4 females; median age 34 years, range 16–49) for peripheral stem cell transplantation using an automated blood cell analyzer. On day 3 (D3) compared with D0, 11 donors with normal laboratory and physical findings showed increases in platelet indices ($\chi^2 = 12.0$, $p = 0.0025$). Furthermore, mean platelet volume (MPV) was significantly increased ($p = 0.04$). Also, platelet count decreased, and platelet distribution width and platelet-large cell ratio were increased, but these were not significant. On the contrary, 1 donor with abnormal laboratory findings who had large platelets (MPV 11.4 fl) before G-CSF administration showed decreases in platelet indices (MPV 10.3 fl) on D3, although platelet count ($18.2 \times 10^4/\mu\text{l}$) decreased after G-CSF administration. G-CSF administration induces an inflammatory process with endothelial

cell activation. This is probably the reason why platelet volume increases after G-CSF use. This is the first report showing that G-CSF administration immediately induces increases in large platelets in peripheral stem cell transplant donors before harvest.

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Introduction

Granulocyte colony-stimulating factor (G-CSF) is widely used in healthy donors for allogeneic peripheral blood stem cell transplantation. However, some cases of thrombosis occurring in elderly donors, possibly because of G-CSF, have also been published [1, 2]. Some data have suggested that G-CSF induces a hypercoagulable state according to the results of platelet aggregation, platelet activation markers and coagulation studies [3, 4]. The mechanism of the effect of G-CSF on platelet activation remains poorly understood. However, platelet activation may cause an accelerated platelet turnover and an increase in larger platelets. Recent studies have shown that an increase in large platelets is a risk factor for acute coronary syndromes [5, 6] and cerebral infarction [7, 8].

Table 1. Minimum, maximum and median platelet counts and platelet indices in 11 normal donors

	PLT, $\times 10^4/\mu\text{l}$	MPV, fl	P-LCR, fl	PDW, fl
D0	15.3–34.1 (24.5)	8.1–10.9 (10.0)	12.6–31.8 (26.6)	9.7–12.8 (12.3)
D1	15.9–30.2 (23.2)	8.2–11.2 (10.0)	13.1–34.2 (24.3)	9.7–13.0 (11.0)
D2	17.4–27.9 (22.0)	9.6–11.4 (10.1)	21.0–29.6 (26.0)	10.3–14.1 (12.3)
D3	16.4–25.3 (21.4)	9.5–11.2 (10.4)*	20.3–35.6 (29.1)	10.7–14.3 (12.9)

Figures in parentheses are medians. * $p = 0.04$.

We hypothesized that platelet size will increase on platelet activation after G-CSF use. To test this hypothesis, we investigated the short-term influence of G-CSF administration on platelet counts and platelet indices in 12 donors for stem cell transplantation using an automated blood cell analyzer.

Subjects and Methods

12 donors (8 males and 4 females; median age 34 years, range 16–49) were included, comprising 4 sons, 6 daughters, 1 mother and 1 brother. Human leukocyte antigen types were all mismatched, except for 1 brother. There was no medical history of note in any donors. Before G-CSF administration, medical history was taken, and physical examination and routine laboratory investigations (liver functions and serum lipids) were performed in all donors. As a result, 1 donor (a 27-year-old male) showed many abnormal findings. His body weight was 75 kg, height was 160 cm, and blood pressure was 160/95 mm Hg. Abdominal echography clearly revealed a fatty liver. Liver functions and other laboratory data were as follows: serum glutamic-oxaloacetic transaminase 27 IU/l, serum glutamic-pyruvic transaminase 46 IU/l, uric acid 7.6 mg/dl, total cholesterol 230 mg/dl, triglycerides 222 mg/dl, and C-reactive protein 0.05 mg/dl. Electrocardiography and urinary examinations were normal. We explained to him the danger of G-CSF administration. The other 11 donors showed completely normal results for the above examinations. Therefore, we segregated this donor from the other 11 donors and evaluated him separately. None had taken drugs including aspirin or aspirin-containing drugs prior to donation. Written informed consent was obtained from each individual before starting the procedure.

G-CSF (lenograstin, Chugai Ltd., Tokyo, Japan, or filgrastin, Kirin Ltd., Tokyo, Japan) was given subcutaneously at a dose of 500 $\mu\text{g}/\text{day}$ to 6 donors (lenograstin) and 600 $\mu\text{g}/\text{day}$ to 6 donors (filgrastin) in 2 daily divided doses for 4 or 5 days. Stem cell apheresis procedures were started in 11 donors on the 4th and in 1 donor on the 5th day after G-CSF administration. Platelet indices were measured before and after G-CSF administration before apheresis. Four blood samples were drawn at different times: day 0 (D0), within 2 h before G-CSF administration; D1,

at 24 h; D2, after 2 days; and D3, 3 days after the first G-CSF administration. Venous blood samples were drawn by venipuncture of the forearm with minimum stasis through a 21-gauge needle into sterile evacuated tubes containing 2K-EDTA. White blood cell, red blood cell and platelet counts, platelet indices (mean platelet volume, MPV; platelet-large cell ratio, P-LCR; platelet distribution width, PDW), hemoglobin level and hematocrit were determined using an automated cell counter (Sysmex NE-8000, Sysmex Ltd., Kobe, Japan) within 2 h after venipuncture.

Fisher's least significant difference post hoc test was used to identify on which day group differences in the 11 normal donors accounted for significant p values. The χ^2 test was used to assess the 11 donors with normal and the 1 donor with abnormal physical and laboratory data.

Results

Changes observed in platelet counts and platelet indices in the donors after G-CSF administration are presented in figure 1. Minimum, maximum and median platelet counts and platelet indices of the 11 normal controls are also shown in table 1. Their significance from D0 to D3 is shown in table 2. On D3 compared with D0, 11 of 12 donors showed decreases in platelet count, excluding 1 donor with normal laboratory findings. Also, all 11 donors with normal laboratory and physical findings showed increases in platelet indices ($\chi^2 = 12.0$, $p = 0.0025$). Furthermore, on D3 compared with D0, MPV was significantly increased ($p = 0.04$), platelet count was decreased, and PDW and P-LCR were increased, but non-significantly (tables 1, 2). On the contrary, the donor with abnormal laboratory findings who showed large platelets (MPV 11.4 fl, P-LCR 36.4% and PDW 14.9 fl) before G-CSF administration showed decreases in platelet indices (MPV 10.3 fl, P-LCR 28.9% and PDW 13.5 fl) on D3, although the platelet count ($18.2 \times 10^4/\mu\text{l}$) decreased after G-CSF administration (fig. 1). There were

Fig. 1. Time course of platelet counts and platelet indices after G-CSF administration in allogeneic transplant donors. P-LCR is the percentage of large platelets defined as the platelet volume exceeding 12 fl. ○ = The case with abnormal laboratory and physical findings; ● = 11 cases showing normal laboratory and physical findings.

Table 2. p values on D0, D1, D2 and D3 for platelet counts and platelet indices in 11 normal donors

	D0 vs. D1	D0 vs. D2	D0 vs. D3	D1 vs. D2	D1 vs. D3	D2 vs. D3
PLT	0.38	0.16	0.09	0.59	0.39	0.74
MPV	0.76	0.25	0.04	0.41	0.08	0.34
P-LCR	0.93	0.29	0.08	0.25	0.07	0.48
PDW	0.70	0.29	0.07	0.50	0.14	0.41

no differences in platelet counts and platelet indices between the groups using lenograstin and filgrastin. The procedure did not alter hemoglobin concentrations in the donors until D3.

Discussion

Decreases in platelet count immediately before apheresis (nonsignificant) and also decreased hemoglobin levels ($p < 0.01$) occurred following G-CSF administration [9]. However, we did not identify decreased hemoglobin levels. Decreases in platelet counts after G-CSF administration without apheresis have been reported [10]. A pre-apheresis platelet decrease was correlated with the total G-CSF dose administered, baseline platelet level and donor age [11]. There was a difference in the frequency of platelet reduction after G-CSF between our and the previous results. This may be due to a difference in donor ages or the development of atherosclerosis as a result of lipidemia or hypertension. Our donors were all young and showed normal laboratory and physiological findings, except for 1 donor who showed decreased platelet indices. Other studies included many aged donors with abnormal laboratory data or no laboratory examination data.

The mechanism of such thrombocytopenia is unclear. Platelets are directly influenced by G-CSF through the G-CSF receptor. This signal may cause platelet aggregation in the presence of agonists, including collagen and/or ADP at local sites in the bloodstream. Granulocytes are also activated by G-CSF *in vivo*. The expression of adhesion molecules may modulate the function of platelets, monocytes and vascular endothelial cells and may enhance cell-to-cell attachment and interaction with co-

agulation factors on the cell surface [12]. Thus, it may lead to a hypercoagulable state. Cella et al. [13] hypothesized that during G-CSF administration, a significant alteration of the endothelium might occur in association with abnormal oxidative stress. G-CSF administration induces an inflammatory process with endothelial cell activation.

We showed that the thrombin-antithrombin complex was positively correlated with platelet indices in patients with aortic aneurysm [14] and ischemic heart disease [15], and that von Willebrand factor antigen and age are also positively correlated with platelet indices in aged normal controls [14]. These findings indicate that thrombin generation due to endothelial degeneration in atherosclerotic diseases and increased von Willebrand factor antigen as a result of endothelial injury caused by aging may consume platelets and accelerate platelet turnover, and large platelets may increase in the peripheral blood. Platelet volume decreases in aortic aneurysms [14] and in ischemic heart disease patients with severe coronary artery disease [15]. The mechanism of platelet volume decrease after G-CSF use in our 27-year-old male with abnormal laboratory data may be the same as that of the above aortic aneurysm and ischemic heart disease patients. This is the first report showing that G-CSF administration immediately induces increases in MPV in peripheral stem cell donors after G-CSF administration, especially on D3.

Conclusion

Platelet volume increases immediately after G-CSF administration, especially on D3. This increase may be a risk factor for thrombosis.

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