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Thrombopoietin therapy increases platelet yields in healthy platelet donors

David J. Kuter, Lawrence T. Goodnough, John Romo, John DiPersio, Randolph Peterson, Dianne Tomita, William Sheridan, and Jeffrey McCullough

The recombinant thrombopoietins have been shown to be effective stimulators of platelet production in cancer patients. It was therefore of interest to determine if one of these, pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF), could be used to increase platelet counts and consequently platelet yields from apheresis in healthy platelet donors. In a blinded, 2-cycle, crossover study, 59 platelet donors were randomized to receive a single subcutaneous injection of PEG-rHuMGDF (1 μg/kg or 3 μg/kg) or placebo and 15 days later undergo platelet apheresis. Donors treated with placebo had a median peak platelet count after PEG-rHuMGDF injection of 248 × 10^9/L compared with 366 × 10^9/L in donors treated with 1 μg/kg PEG-rHuMGDF and 602 × 10^9/L in donors treated with 3 μg/kg PEG-rHuMGDF. The median maximum percentage that platelet counts increased from baseline was 10% in donors who received placebo compared with 70% in donors who received 1 μg/kg and 167% in donors who received 3 μg/kg PEG-rHuMGDF. There was a direct relationship between the platelet yield and the preapheresis platelet count: Placebo-treated donors provided 3.8 × 10^{11} (range 1.3 × 10^{11}-7.9 × 10^{11}) platelets compared with 5.6 × 10^{11} (range 2.6 × 10^{11}-12.5 × 10^{11}) or 11.0 × 10^{11} (range 7.1 × 10^{11}-18.3 × 10^{11}) in donors treated with 1 μg/kg or 3 μg/kg PEG-rHuMGDF, respectively. Substandard collections (<3 × 10^{11} platelets) were obtained from 26%, 4%, and 0% of the placebo, 1 μg/kg, and 3 μg/kg donors, respectively. No serious adverse events were reported; nor were there events that met the criteria for dose-limiting toxicity. Thrombopoietin therapy can increase platelet counts in healthy donors to provide a median 3-fold more apheresis platelets compared with untreated donors. (Blood. 2001;98:1339-1345)

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Introduction

Platelet transfusions are essential to prevent morbidity and mortality in patients who are severely thrombocytopenic. Platelets for transfusion are currently obtained either by fractionation of whole blood or by platelet apheresis.[1-4] Fractionation of a unit of donated whole blood produces a unit of platelet concentrate that should contain at least 5.5 × 10^{10} platelets,[5] and 4 to 6 such platelet concentrates are typically combined for one platelet transfusion in an adult patient.

Increasingly, platelets are being obtained by platelet apheresis, and these account for more than half [4,6] of the platelets currently transfused. The average yield of platelets collected by platelet apheresis is approximately 4.2 × 10^{11} platelets.[1,7,8] The platelet yield depends on many factors, but with current automated technologies, the donor's preapheresis count, total blood volume, and the duration of the procedure are the predominant determinants.[7,8] Donor comfort and convenience limit the last parameter.

Thrombopoietin is the endogenous agonist of the cytokine receptor, Mpl, and is the primary regulator of platelet production. Several recombinant thrombopoietins have been under clinical development. One of these, pegylated recombinant human megakaryocyte growth and development factor[9] (PEG-rHuMGDF), is an effective thrombopoietic agent in patients with cancer.[10-12] In addition, platelets produced under stimulation with PEG-rHuMGDF appear to retain normal hemostatic function as assessed by in vitro assays.[4,11,13]

We therefore designed a clinical trial to evaluate the dose response and safety of PEG-rHuMGDF to increase the yield of platelets collected by apheresis from healthy donors. In a companion study, we examined the safety and efficacy of prophylactic transfusion of these platelet products into thrombocytopenic recipients (see accompanying article by Goodnough et al,[14] page 1346).

Materials and methods

Donor population

Platelet apheresis donors at participating study centers who had donated at least twice in the previous 12 months were eligible for participation. Donors were also required to be 50 years old or younger; have no personal or family history of cardiovascular or thrombotic disease before age 50 years; have a normal platelet count (ie, 150 × 10^9/L to 450 × 10^9/L) at each of the 2 previous donations; not smoke cigarettes or take oral contraceptives; not be morbidly obese; not be participating in a directed-blood donor program; and not be pregnant or breast-feeding.

The institutional review boards of the participating centers approved the protocol, and all donors gave written informed consent before study entry in accordance with the Helsinki protocol.

Study design and laboratory monitoring

The objective of this study was to determine the clinically effective dose of PEG-rHuMGDF: no dose-limiting toxicity, no more than 2 of 10 donors

From Massachusetts General Hospital, Boston; Washington University, St Louis, MO; University of Minnesota, Minneapolis; and Amgen, Thousand Oaks, CA.

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Reprints: David J. Kuter, Hematology/Oncology Unit, COX 640, Massachusetts General Hospital, 100 Blossom St, Boston, MA 02114; e-mail: kuter.david@mgh.harvard.edu.

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with a platelet count more than 1000×10^9/L, and 80% or more of donors with a doubling of platelet count compared with baseline by the planned day of apheresis (day 15). A blinded, 2-cycle, crossover, dose-escalation design was used. At least 25 eligible donors were to be randomized to each dose of PEG-rHuMGDF studied. Donors were randomized in a 2:2:1 allocation to receive a single subcutaneous injection of either PEG-rHuMGDF in the first cycle followed by placebo in the second cycle (10 donors), placebo in the first cycle followed by PEG-rHuMGDF in the second cycle (10 donors), or placebo in both cycles (5 donors).

The first injection of blinded study drug was given on the first day of the first cycle, and the second injection of blinded study drug was given 28 days later. Platelet apheresis was done on day 15 of each cycle of study drug administration.

Hematology values (platelet count, white blood cell count with differential, and hematocrit) were obtained in each cycle on days 1 (before study drug administration), 6, 9, 12, 15 (before and after platelet apheresis), and 22. Serum chemistries, including ferritin, were determined at baseline and on the first day of each cycle. Samples for measurement of antibodies to PEG-rHuMGDF[15] were drawn in each cycle before administration of the study drug, at the end of the study (day 29 of the second cycle), and 3 and 6 months later.

At one site, platelet function tests were performed 2 to 4 hours after collection of the apheresis products. The platelet concentration was adjusted to 300×10^9/L and the extent of aggregation measured upon exposure to 8 different concentrations of adenosine diphosphate (ADP) (0-40 μM).[16] The extent of spontaneous aggregation and the dose of ADP that produces 50% of maximal aggregation were determined.

The dose of PEG-rHuMGDF was escalated to the next level as long as no dose-limiting toxicities were seen, and a doubling of the platelet count compared with baseline was seen in fewer than 80% of the cycles in the first 10 donors treated with PEG-rHuMGDF. Dose escalation stopped when a clinically effective dose was obtained. Any donor found to have a platelet count 1000×10^9/L or higher was to undergo early platelet apheresis.

Platelet apheresis procedure

One platelet apheresis was performed on day 15 of each cycle of the study using a single-arm procedure with the Cobe Spectra (Cobe BCT, Lakewood, CO). Platelet apheresis was done for the initial 66 collections without the leukocyte reduction system and thereafter with the leukocyte reduction system for the remaining 44 collections. The volume of blood processed was standardized as 85% of the donor's estimated total blood volume as calculated by a Cobe Spectra software algorithm based on donor sex, height, weight, and hematocrit. All platelet apheresis procedures were conducted at a rotor speed of 2400 rpm and were also standardized across all institutions for other machine variables. After collection, products were split into separate platelet storage bags per institutional protocol and stored at 22°C on oscillating racks.

Study drug

PEG-rHuMGDF is formulated as a sterile, clear, preservative-free liquid.[10] Placebo and study drug (250 and 500 μg/mL) were packaged in identical vials. Investigators, all study staff, and study monitors were blinded to study drug assignment.

Statistical analysis

The effect of the study drug on continuous variables was analyzed by dose group (0 [placebo] vs 1 μg/kg vs 3 μg/kg PEG-rHuMGDF), treatment arm (placebo in both cycles vs placebo in cycle 1 followed by PEG-rHuMGDF in cycle 2 vs PEG-rHuMGDF in cycle 1 followed by placebo in cycle 2), cycle (cycle 1 PEG-rHuMGDF vs cycle 2 PEG-rHuMGDF within a dose group), and appropriate interaction terms using analysis of variance methods. Effects of treatment arm and interaction terms were not found to be statistically significant. Thus, the results are presented by dose group only. The effect of PEG-rHuMGDF on dichotomous or categoric variables was analyzed using either the Fisher exact test or the χ^2 test.

Pharmacodynamic variables, such as median daily platelet count, maximum platelet count, and fold change in platelet count compared to baseline, are summarized using donor-cycle as the unit of measurement. Variables associated with the apheresis procedure, platelet yield, platelet product variables (eg, concentration), and change in platelet count following the apheresis procedure were analyzed for the subset of donors who completed the apheresis.

Results

Donor population

Fifty-nine platelet donors were randomized in this study. Most of the donors were male and Caucasian. Treatment groups were well balanced with respect to other demographic characteristics and baseline platelet counts (Table 1). All donors randomized in this study received their first injection of the study drug, and all were evaluable for safety analysis. Two donors were withdrawn before the second cycle because of unrelated medical conditions (borderline positive test for hepatitis C, prostate biopsy for benign prostatic hypertrophy).

Platelet count response

Figure 1 shows the platelet count response by study drug and drug dose. The 59 donors had a total of 116 donor-cycles of platelet count data (70 cycles with placebo, 23 cycles with 1 μg/kg PEG-rHuMGDF, and 23 cycles with 3 μg/kg PEG-rHuMGDF). Among donors administered placebo, the platelet count varied little during days 1 to 15 before platelet apheresis, decreased below baseline after platelet apheresis as expected,[7] and recovered to baseline levels by day 22. In donors treated with PEG-rHuMGDF, the platelet count was increased compared with baseline by day 6, reached a maximum on day 12, and decreased slightly by the time of platelet apheresis on day 15. After platelet apheresis on day 15, the platelet count declined but still remained above baseline until day 28. There was no effect of PEG-rHuMGDF on the hemoglobin, white blood cell count, or neutrophil count, and these parameters did not change after platelet apheresis.

The median baseline platelet count was similar in all 3 dose groups (Table 2). The median maximum platelet count in donors

Table 1. Donor demographics

	Placebo (n = 13)	PEG-rHuMGDF 1 μg/kg (n = 23)	PEG-rHuMGDF 3 μg/kg (n = 23)
Gender			
Male, no. (%)	10 (73)	20 (87)	20 (87)
Female, no. (%)	3 (27)	3 (13)	3 (13)
Ethnicity			
White, no. (%)	13 (100)	20 (87)	23 (100)
Other, no. (%)	0 (0)	3 (13)	0 (0)
Age, y			
Median	41	41	41
Range	26-48	27-50	27-50
Weight, kg			
Median	82	84	91
Range	68-123	70-121	73-110
Height, cm			
Median	177	178	178
Range	160-188	163-201	154-191
Baseline platelet count, $\times 10^9$/L*			
Median	232	230	227
Range	168-341	169-328	161-366

*Baseline platelet count taken on the first day of the first cycle.

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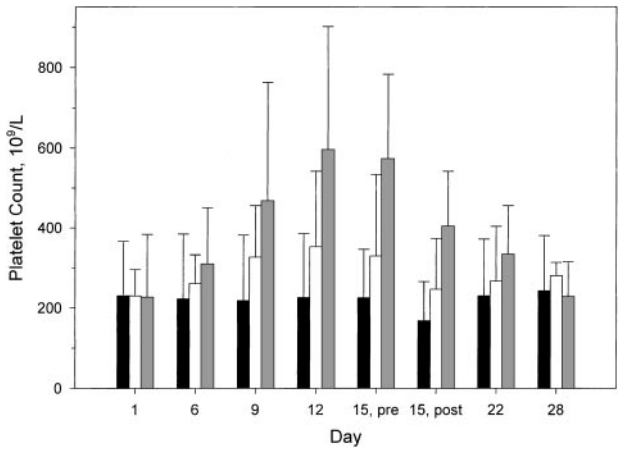

Figure 1. The platelet count rises in a dose-dependent manner following the administration of PEG-rHuMGDF to apheresis donors. The response of the donor median platelet count (+ SD) to placebo (■), 1 μg/kg PEG-rHuMGDF (□), or 3 μg/kg PEG-rHuMGDF (▨) administration. Platelet counts in donors were obtained on day 1 just prior to injection, on the indicated days thereafter, before (15, pre) and after (15, post) platelet apheresis.

treated with PEG-rHuMGDF increased in a dose-related manner. Subjects treated with placebo had a median peak platelet count of 248×10^9/L, compared with 366×10^9/L in subjects treated with 1 μg/kg PEG-rHuMGDF and 602×10^9/L in subjects treated with 3 μg/kg PEG-rHuMGDF ($P < .001$). The highest platelet count achieved was 901×10^9/L. No donor-administered placebo had more than a 2-fold increase in platelet count compared with 22% of donors administered 1 μg/kg PEG-rHuMGDF and 95% of donors treated with 3 μg/kg PEG-rHuMGDF.

There was no correlation between the baseline platelet count and the maximum platelet count attained in any of the treatment groups. At baseline, women donors had a 24% higher median platelet count than men donors (272×10^9/L vs 219×10^9/L, $P < .001$). This difference is not unexpected[7] and did not affect response to PEG-rHuMGDF (data not shown). It may be related to the difference ($P < .001$) in median serum ferritin between men (56 ng/mL, range 5-190) and women (21 ng/mL, range 8-113) because iron deficiency is associated with an increase in the platelet count.[17]

Prior to the scheduled apheresis on day 15, the platelet count for both PEG-rHuMGDF groups was 10% lower (Table 3) than the maximal platelet count but still showed the same dose-dependent increase seen for the maximal platelet count changes.

Table 2. Baseline and maximum platelet count by treatment group

	Placebo (n = 70 cycles)	PEG-rHuMGDF 1 μg/kg (n = 23 cycles)	PEG-rHuMGDF 3 μg/kg (n = 23 cycles)	*P*
Baseline, × 10^9/L*				
Median	230	230	227	NS
Range	165-366	170-296	161-384	
Maximum, × 10^9/L†				
Median	248	366	602	< .001
Range	180-386	231-542	438-901	
Maximum fold change from baseline				
Median	1.1	1.7	2.7	< .001
Range	1.0-1.8	1.1-2.4	2.1-3.6	
Category of maximum platelet count, × 10^9/L‡				
Less than 450	70 (100)	18 (78)	1 (4)	
450-750	0 (0)	5 (22)	15 (65)	< .001
More than 750	0 (0)	0 (0)	7 (30)	

NS indicates nonsignificant.
*Baseline platelet count taken as the day 1 value of each cycle.
†Maximum platelet count after day 1 and prior to apheresis.
‡Number (%) of donors with the indicated maximum platelet count.

Platelet apheresis

The 59 donors had a total of 110 completed apheresis procedures (65 completed procedures following administration of placebo, 23 following 1 μg/kg PEG-rHuMGDF, and 22 following 3 μg/kg PEG-rHuMGDF). Six donors had an incomplete platelet apheresis, which was due to venous infiltration (4), citrate reaction (1), or low platelet count (1); and 2 donors were deferred from a second cycle of platelet apheresis because of unrelated medical conditions.

There was a high degree of compliance with the standardized platelet apheresis protocol as shown by the correlation between the actual and predicted blood volume processed ($r = 0.94$) and between the actual and predicted volume of platelet-rich plasma collected ($r = 0.89$). The duration of the apheresis procedure was not significantly different in the 3 treatment groups (median duration of 95, 92, and 100 minutes for the placebo, 1 μg/kg, and 3 μg/kg groups, respectively).

The platelet yield from apheresis (Table 3) was 3.8×10^{11} in donors treated with placebo, compared with 5.6×10^{11} in donors treated with 1 μg/kg PEG-rHuMGDF and 11.0×10^{11} in donors treated with 3 μg/kg PEG-rHuMGDF ($P < .001$). When the actual platelet yield was compared with the yield predicted by the Cobe Spectra algorithm, there was a high degree of linear correlation overall ($r = 0.88$).

Baseline, maximum, preapheresis, and postapheresis platelet counts were examined for correlation to platelet yield. There was no correlation with the baseline platelet count, but both the maximum platelet count (approximately day 12, $r = 0.91$) and the preapheresis platelet count (day 15, $r = 0.92$) were highly correlated with platelet yield (Figure 2). The postapheresis platelet count was higher in donors treated with PEG-rHuMGDF than in donors administered placebo (Table 3), but the postapheresis platelet count was approximately the same percentage of the preapheresis count (73% to 75%) for all 3 cohorts. As expected, the median absolute decrease in the platelet count after apheresis was larger in donors treated with PEG-rHuMGDF (-87×10^9/L and -157×10^9/L for

Table 3. Platelet apheresis yield by treatment group

	Placebo (n = 65)	PEG-rHuMGDF 1 μg/kg (n = 23)	PEG-rHuMGDF 3 μg/kg (n = 22)	*P*
Preapheresis platelet count, × 10^9/L*				
Median	231	330	544	< .001
Range	155-346	208-533	341-789	
Postapheresis platelet count, × 10^9/L*				
Median	168	247	405	< .001
Range	121-266	163-347	278-542	
Platelet yield, × 10^{11}				
Median	3.8	5.6	11.0	< .001
Range	1.3-7.9	2.6-12.5	7.1-18.3	
Collection efficiency†				
Median	0.44	0.49	0.53	< .001
Range	0.18-0.72	0.13-0.58	0.45-0.64	

*On day 15.
†Number of platelets collected/[([preapheresis count + postapheresis count]/2) × blood volume processed].

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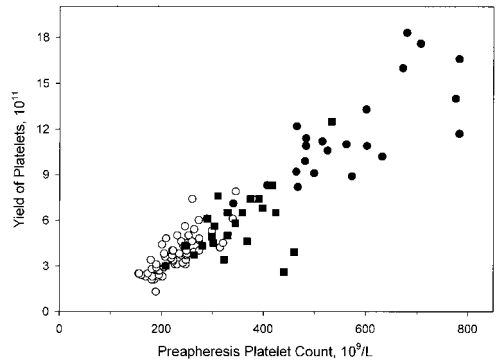

Figure 2. Platelet apheresis yield is directly proportional to the donor preapheresis platelet count. The relationship between the preapheresis platelet count on day 15 and the platelet apheresis yield is shown for donors treated with placebo (○, n = 65), 1 μg/kg PEG-rHuMGDF (■, n = 23), or 3 μg/kg PEG-rHuMGDF (●, n = 22); $r = 0.92$.

the 1 μg/kg and 3 μg/kg cohorts, respectively) than in donors given placebo (-56×10^9/L), and this decrease showed a strong correlation with the platelet yield.

The collection efficiency is a measurement of how well the cell separator removes platelets from the blood that it has processed. For the cell separator used in these studies, 45% to 60% (efficiency = 0.45-0.60) of the platelets that pass through the separator are routinely collected.[18] Because the technical ability of the cell separator used in this study had rarely been tested at such elevated platelet counts, collection efficiency was measured for all 3 treatment groups. There was actually an increase in the collection efficiency ($P < .001$) at the higher platelet counts (Table 3). Platelets were collected at a median rate of 4×10^9/min (range 1.3×10^9/min to 7.5×10^9/min), 5.5×10^9/min (range 2.5×10^9/min to 12×10^9/min), and 10.9×10^9/min (range 7.1×10^9/min to 17.1×10^9/min) for the placebo, 1 μg/kg, and 3 μg/kg cohorts, respectively.

Because a yield of 3×10^{11} platelets in more than 75% of collections is considered the minimum standard for apheresis platelet units,[5,7] the ability of the products in this study to meet this standard was analyzed (Table 4). Among donors given placebo, 17 (26%) of 65 platelet apheresis collections had fewer than 3×10^{11} platelets, compared with 1 (4%) of 23 for donors given 1 μg/kg PEG-rHuMGDF and none (0%) of 22 for donors given 3 μg/kg PEG-rHuMGDF. In contrast, triple (9×10^{11} to $<12 \times 10^{11}$ platelets) or quadruple ($\geq 12 \times 10^{11}$ platelets) products were obtained 82% of the time in the 3 μg/kg cohort but in none of the placebo group. The relatively high rate of platelet collections with fewer than 3×10^{11} platelets in the placebo group is due to the fixed blood volume processed in the protocol; in practice, larger blood volumes would have been processed and the yields somewhat higher.

The ability of platelets to remain viable after storage at 22°C for up to 5 days is dependent on the platelet concentration and volume of platelet-rich plasma in the storage bag. The recommended storage concentration with the Cobe system is 1.0×10^9/mL to 2.1×10^9/mL, and the recommended volume is 100 to 400 mL.[19] The distribution of platelet storage concentration (platelet yield/volume of anticoagulated plasma collected) and storage volume were therefore determined for the products in this study (Table 4). All storage volumes per bag were within the recommended range because larger volume products were split for storage. Among the platelet products collected from donors administered placebo, 9% were less than 1.0×10^9/mL and none were more than 2.1×10^9/mL. Among donors treated with 1 μg/kg PEG-rHuMGDF, 9% ofproducts were less than 1.0×10^9/mL, and 4% were more than 2.1×10^9/mL. Among donors treated with 3 μg/kg PEG-rHuMGDF, no products were less than 1.0×10^9/mL; however, 32% of products were between 2.1×10^9/mL and 2.8×10^9/mL. As expected, there was a strong correlation between platelet yield and platelet storage concentration ($r = 0.86$). The volume of platelet-rich plasma collected increased with the platelet yield (Table 4) but in no case exceeded the standards for volume of plasma removed from individual donors.[5]

Laboratory monitoring

End-of-study measurements of all biochemical parameters showed no changes from baseline values. Donors were monitored during both treatment cycles and thereafter at 3 months and 6 months for the development of antibodies to PEG-rHuMGDF. None were detected in any donor. None of the subjects became thrombocytopenic (platelets less than 100×10^9/L). There was also no change in the platelet count 6 months after the end of the study. In placebo-treated donors the median baseline platelet count was 227×10^9/L (range 168×10^9/L-341×10^9/L), and 6 months after the conclusion of the study the median platelet count was 207×10^9/L (range 164×10^9/L-323×10^9/L). For the 1-μg/kg and 3-μg/kg subjects, the median baseline platelet count was 227×10^9/L (range 150×10^9/L-366×10^9/L), and 6 months after the conclusion of the study the median platelet count was 222×10^9/L (range 146×10^9/L-321×10^9/L).

Platelet aggregation testing was performed on 35 of the apheresis products. There was no statistically significant difference in the ADP median effective concentrations (medians 2.26, 2.12, 3.86 μM) or the extent of spontaneous aggregation (medians 5.0%, 3.6%, 5.7%) for the placebo (n = 21), 1 μg/kg (n = 6), or 3 μg/kg (n = 8) PEG-rHuMGDF products, respectively.

Table 4. Analysis of platelet apheresis products by treatment group

	Placebo (n = 65)	PEG-rHuMGDF 1 μg/kg (n = 23)	PEG-rHuMGDF 3 μg/kg (n = 22)	*P*
Category of platelet yield, $\times 10^{11}$*				
Less than 3	17 (26)	1 (4)	0 (0)	
3 up to 6	43 (66)	12 (52)	0 (0)	
6 up to 9	5 (8)	9 (39)	4 (18)	< .001
9 up to 12	0 (0)	0 (0)	11 (50)	
At least 12	0 (0)	1 (4)	7 (32)	
Volume platelet-rich plasma, mL				
Median	288	422	576	< .001
Range	163-513	227-737	446-711	
Platelet concentration, $\times 10^9$/mL†				
Median	1.2	1.4	2.0	< .001
Range	0.56-2.0	0.66-2.3	1.3-2.8	
Category of platelet concentration, $\times 10^9$/mL)‡				
Less than 1.0	6 (9)	2 (9)	0 (0)	
1.0-2.1	59 (91)	20 (87)	15 (68)	< .001
More than 2.1	0 (0)	1 (4)	7 (32)	

*Number (%) of products containing the indicated amount of platelets.
†Platelet concentration in platelet apheresis product.
‡Number (%) of products containing the indicated range of platelet concentration.

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Safety

The incidence of adverse events is shown in Table 5. All events except one (a severe headache in a placebo-treated donor) were reported as mild or moderate in severity, and no serious events were reported. The most frequently reported adverse event in donors exposed to PEG-rHuMGDF was headache, and all resolved either spontaneously or with a pain medication (acetaminophen or other nonprescription pain product). Headaches were reported more frequently in the 3 μg/kg dose group, but this did not reach statistical significance. Circumoral paresthesia, a common effect of citrate anticoagulant used during apheresis, was noted during procedures in 4% of PEG-rHuMGDF–treated and 9% of placebo-treated donors.

Discussion

For more than 30 years, platelet transfusions have been the primary therapy for patients with thrombocytopenia. Whether the use of new thrombopoietic growth factors such as recombinant thrombopoietin or interleukin-11 will impact this need is unclear.[4] Over the past decade there has been a shift away from pooled random donor platelet concentrates to single-donor apheresis platelets.[4,6] Although usually more expensive than comparable doses of platelet concentrates, the reason for this shift has been the perception that single-donor platelets are a better platelet product due to the reduction in the number of donors to which the patient is exposed and the consequent reduction in transfusion-transmitted disease, bacterial contamination,[2] febrile transfusion reactions,[20] and possibly alloimmunization.[21] It is estimated that currently more than 50% of platelets are produced by platelet apheresis[4,6] despite great variability among geographic regions.

Platelets are a perishable commodity that are outdated after 5 days of storage at room temperature, and this results in the continuous need to replace this valuable resource. Methods to freeze platelets are available but are complex, costly, and not widely used. Therefore, efforts to improve platelet apheresis yields are a valuable strategy for supplementing the national blood platelet supply. Because the key variable in platelet apheresis yield is the donor platelet count, we have studied whether increasing the donor platelet count with thrombopoietin therapy might be an effective and safe approach to increasing the yield of platelet apheresis.

We have shown that a single subcutaneous injection of PEG-rHuMGDF in healthy platelet donors produces a dose-dependent increase in the platelet count that is maximal on day 12 and then declines to baseline by day 28. This time course is identical to those previously seen in cancer patients given PEG-rHuMGDF prior to chemotherapy.[12] Although most of the donors were Caucasian and male, it is unlikely that the responses obtained reflect ethnic or gender biases. Similar responses have been seen in women undergoing chemotherapy[22] and in Japanese subjects. There was large variability in the maximal platelet response between donors at either PEG-rHuMGDF dose, but the 3 μg/kg dose fulfilled the study objectives and is the clinically effective dose in that all donors had a platelet count increase of at least 2-fold and none exceeded 1000×10^9/L. This is comparable to the clinically effective 3- to 5-μg/kg dose found in cancer patients undergoing chemotherapy.[10,11]

The increased platelet count resulted in increased platelet apheresis yields. Although this response had been predicted by previous studies over a narrow range of donor platelet counts,[8,23] our studies have confirmed and extended this principle over an extensive range of donor platelet counts. The linearity of the response of platelet yield to platelet count (Figure 2) showed no evidence of reaching a plateau even at platelet counts up to 901×10^9/L. Because equivalent amounts of blood were processed in all 3 treatment groups (85% of total blood volume), the increased platelet yield from the PEG-rHuMGDF–treated donors was the result of increased platelet clearance by the cell separator at the higher platelet counts. As demonstrated by the improved collection efficiency, there appears to be no technical limitation of the cell separator at these elevated platelet counts. Even higher platelet yields might be obtained by harvesting donors at their maximal platelet count on day 12, rather than at day 15, and/or performing multiple apheresis procedures during the period of elevated platelet levels. For example, the number of platelets collected (11×10^{11}) at the 3 μg/kg dose accounted for only 37% of those potentially available for collection had the apheresis been repeated until the postapheresis count reached the value observed in the placebo-treated donors (168×10^9/L). Furthermore, the improved platelet yield at the higher platelet counts reduced the number of substandard products and markedly increased the potential number of apheresis products that could be split. Platelet aggregation testing showed that the PEG-rHuMGDF–mobilized platelets also had normal function.

The greatly increased platelet yields available after thrombopoietin therapy present potential advantages. For the blood center these include the ability to provide increased platelet inventory from perhaps a stable or even declining donor base and possibly at a reduced unit cost. Each high-yield platelet product could be divided into multiple products each containing the same number of platelets as existing products. Alternatively, each high-yield product could be used as a single product. This method may be particularly helpful in providing platelets from donors with certain HLA types or from directed donors due to the possibility of

Table 5. Incidence of adverse events reported in all donor cycles

	Placebo n = 70	All PEG-rHuMGDF n = 46	PEG-rHuMGDF 1.0 μg/kg/d n = 23	PEG-rHuMGDF 3.0 μg/kg/d n = 23
No. of adverse events reported in any donor cycle (%)	28 (40)	18 (39)	7 (30)	11 (48)
Specific adverse events reported in at least 4% of all cycles, no. (%)				
Headache	5 (7)	8 (17)	2 (9)	6 (26)
Paresthesia	6 (9)	2 (4)	0 (0)	2 (9)
Gastrointestinal (nausea, diarrhea, pain)	5 (7)	3 (7)	1 (4)	2 (9)
Apheresis access problems	4 (6)	2 (4)	1 (4)	1 (4)
Upper respiratory tract congestion	3 (4)	2 (4)	2 (9)	0 (0)
Upper respiratory infection	4 (6)	1 (2)	1 (4)	0 (0)
Apheresis access site ecchymosis	4 (6)	1 (2)	0 (0)	1 (4)

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multiple donations from one such donor over a period of several days. Because a little more than one third of the platelets available for apheresis at the 3 μg/kg dose are actually removed by a single apheresis procedure, it is possible that repetitive apheresis procedures over several days would be desirable in some donors.

For the donor, potential advantages include a reduced likelihood of providing a substandard donation and, possibly, shorter platelet apheresis procedures. This latter aspect may be especially important for platelet donors who voluntarily donate multiple times a year. No donor dropped out of this study because of the inconvenience of visiting the donor center for the PEG-rHuMGDF injection.

For the recipient, a larger platelet dose may translate into a reduced need for subsequent transfusions, a longer interval between transfusions, and fewer donor exposures. This approach was studied in a companion protocol in which thrombocytopenic patients[14] were transfused with the platelets collected in this study.

Before recombinant thrombopoietin-stimulated platelet donation can be routinely employed, 2 issues must be addressed. The first is whether the thrombopoietin-mobilized platelets are effective upon transfusion. Platelets are normally stored for up to 5 days at room temperature at a concentration of 1.0×10^9/mL to 2.1×10^9/mL. The high platelet concentration obtained in the PEG-rHuMGDF products (32% more than 2.1×10^9/mL) may adversely affect platelet viability during storage. However, in a companion study[14] we show that the PEG-rHuMGDF–mobilized platelets have normal posttransfusion recovery and are at least as effective as platelets from placebo-treated donors when transfused into thrombocytopenic recipients.

The second issue is whether repetitive doses of recombinant thrombopoietin should be administered to healthy volunteers. Although only a relatively small number of donors were treated with PEG-rHuMGDF, the only significant adverse event was headache, which has also been observed commonly in donors given met-rHuG-CSF (recombinant methionyl human granulocyte colony-stimulating factor) to mobilize neutrophils or hematopoietic stem cells.[24,25] In reported studies in a much larger number of cancer patients, PEG-rHuMGDF has not been associated with any significant adverse event such as thrombosis, even at platelet counts in excess of 1000×10^9/L.[10-13] However, the clinical development of PEG-rHuMGDF has been discontinued in the United States because in another study 4% of healthy volunteers who received more than one dose of PEG-rHuMGDF developed neutralizing antibodies to endogenous thrombopoietin with consequent thrombocytopenia.[26,27] None of the apheresis donors described herein received more than one injection of PEG-rHuMGDF, and none developed antibodies to thrombopoietin or had any persistent blood count abnormality. Whether a future administration of a recombinant thrombopoietin might cause antibody formation in these subjects is uncertain. All available participants in this study have been informed of the antibody issue and have been enrolled in an ongoing follow-up study.

In summary, our study showed that thrombopoietin therapy is effective in increasing the platelet count and platelet yield in platelet apheresis donors. If another recombinant thrombopoietin, thrombopoietin peptide mimetic,[28] or nonpeptidyl receptor agonist[29] can be developed that lacks the antigenicity of the PEG-rHuMGDF used in these studies, thrombopoietin-stimulated platelet donation may have a significant role in enhancing platelet inventory for health care delivery.

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