Brief report

Effects of pegylated recombinant human megakaryocyte growth and development factor in patients with idiopathic thrombocytopenic purpura

Shosaku Nomura, Kazuo Dan, Tomomitsu Hotta, Kingo Fujimura, and Yasuo Ikeda

We conducted a phase 1-2 clinical trial to evaluate the effect of pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF) in patients with chronic idiopathic thrombocytopenic purpura (ITP) refractory to standard therapy who had platelet counts below $30 \times 10^9/L$. Four patients received PEG-rHuMGDF ($0.5 \mu g/kg$ of body weight per day) by daily intravenous injection for up to 7 days. Administration of PEG-rHuMGDF increased platelet counts in 3 patients. A striking thrombocytosis occurred in 2 patients, whose platelet counts were elevated to more than $700 \times 10^9/L$ a week after the last administration of PEG-rHuMGDF and returned to baseline levels within 4 to 6 weeks. Before the platelet peak, the percentage of reticulated platelets increased transiently in 3 patients tested, including one patient who had no response. Bleeding episodes decreased after the start of PEG-rHuMGDF therapy. These results suggest that PEG-rHuMGDF might have a clinical benefit in ameliorating thrombocytopenia associated with ITP.

© 2002 by The American Society of Hematology

Introduction

Idiopathic thrombocytopenic purpura (ITP) is characterized by thrombocytopenia, increased levels of platelet-associated immunglobulin, and normal to increased numbers of megakaryocytes. The mechanisms of thrombocytopenia and production of antiplatelet antibodies have been investigated extensively. In addition to markedly shortened platelet survival, impaired platelet production is also responsible for thrombocytopenia in ITP. Various therapeutic strategies are used for treatment of patients with ITP, including corticosteroids, danazol, immunosuppressive agents, and splenectomy. High-dose intravenous immunoglobulin causes a transient increase in platelet counts in most patients with ITP. Because of the heterogeneity of the disease, however, approximately 20% of cases are refractory to these treatments. A thrombopoietic cytokine is a candidate for a new medical treatment in some situations. In a recent pilot trial in patients with refractory ITP, however, recombinant human interleukin 11 was not effective.

Thrombopoietin (TPO) is the primary physiologic regulator of platelet production. Initial clinical trials indicated that pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF), a nonglycosylated, truncated form of human TPO modified with polyethylene glycol, potently stimulates platelet production before chemotherapy in patients with cancer. Studies in healthy human volunteers showed that in addition to increasing platelet production, PEG-rHuMGDF increases the number and ploidy of marrow megakaryocytes without influencing platelet function and viability. PEG-rHuMGDF was also used in healthy platelet donors to increase platelet counts and, consequently, platelet yield from apheresis. A median of 3-fold more apheresis platelets were obtained after administration of PEG-rHuMGDF. However, a small but significant proportion of subjects in these studies had development of neutralizing antibodies to endogenous TPO that resulted in thrombocytopenia. Further clinical studies have been performed to assess the therapeutic effect of PEG-rHuMGDF on platelet recovery in patients with cancer receiving myelosuppressive chemotherapy as well as in patients with severe thrombocytopenia associated with aplastic anemia and myelodysplastic syndrome.

In patients with cancer who have received myelosuppressive chemotherapy, serum TPO levels increased during the period of thrombocytopenia and then decreased as counts of circulating platelets increased. In patients with ITP, however, serum TPO levels were normal or only slightly elevated in spite of a marked thrombocytopenia. These findings, along with the observation of normal or increased numbers of megakaryocytes in the bone marrow, suggest that exogenously administered PEG-rHuMGDF might further stimulate megakaryocyteopoiesis and correct thrombocytopenia in patients with ITP. We here describe the first clinical trial of PEG-rHuMGDF in patients with chronic ITP refractory to corticosteroid therapy, splenectomy, or both.

Study design

Patients with chronic ITP refractory to corticosteroid therapy, splenectomy, or both and baseline platelet counts below $30 \times 10^9/L$ during the 3 months before registration were eligible for this study. Patients also met the following criteria: more than 18 but under 70 years of age and an Eastern Cooperative Oncologic Group performance status of 0, 1, or 2. Exclusion dates of manuscript receipt and publication are listed on the first page of each article. Reprints: Yasuo Ikeda, Division of Hematology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan; e-mail: yikeda@sc.ltc.keio.ac.jp.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 U.S.C. section 1734.
criteria included serious bleeding, thrombocytopenia associated with systemic lupus erythematosus, infection with human immunodeficiency virus, splenomegaly, cyclic thrombocytopenia, and previous history of vascular disease or thromboembolism, or marked heart, lung, liver, or renal impairment. All patients gave written informed consent to participation before treatment. The study was approved by the ethics committees of the participating hospitals.

The study was designed as an open-labeled, cohort-sequential, dose-escalation phase 1-2 clinical trial using doses ranging from 0.5 to 10.0 μg/kg of body weight per day of PEG-rHuMGDF (KNR9000; Kirin Brewery Company, Tokyo, Japan). Patients received daily intravenous administration of PEG-rHuMGDF for up to 7 days until platelet counts exceeded 100 × 10^9/L.

Complete blood counts and mean platelet volume (MPV) were measured before treatment, 3 times weekly during the first 2 weeks, and once at 3, 4, 5 and 7 weeks after the start of PEG-rHuMGDF administration. Reticulated platelets were assayed by using auramine O, a fluorescent dye, at one selected site (Keio University), as described previously. Serum was collected for assay of antibodies against PEG-rHuMGDF and nonpegylated rHuMGDF before treatment and at 2 weeks, 4 weeks, 3 months, and 6 months after the final injection of PEG-rHuMGDF. Patients were closely monitored for adverse effects throughout the study.

Results and discussion

Four patients (Table 1) were given PEG-rHuMGDF at a dose of 0.5 μg/kg per day for up to 7 days. This dose was the lowest effective dose in healthy volunteers receiving a single intravenous injection (J. Azuma et al, unpublished results, 1996). The 7-day treatment with PEG-rHuMGDF was completed in patients 1 and 3. However, patient 2 received only a single injection and patient 4 received PEG-rHuMGDF for 6 days (Figure 1).

As shown in Figure 1, patients 2, 3, and 4 had a response to PEG-rHuMGDF therapy. In patients 3 and 4, daily administration of PEG-rHuMGDF dramatically increased platelet counts, which reached a maximum level of 747 × 10^9/L on day 15 in patient 3 and of 821 × 10^9/L on day 14 in patient 4. Such a delayed platelet response likely reflects the mechanism of action of PEG-rHuMGDF, which stimulates megakaryocyte progenitor cells rather than mature megakaryocytes. Even in patient 2, who received only one dose of PEG-rHuMGDF, platelet counts increased up to 103 × 10^9/L on day 10. Patient 1, however, had no platelet response to PEG-rHuMGDF. Although Harker previously observed increased megakaryocytopenia and platelet turnover in patients with ITP, which might have predicted an ineffective response to PEG-rHuMGDF in our patient 1, a substantial increase in platelet counts was clearly observed in 3 of our 4 patients, thereby suggesting a potent stimulation of thrombopoiesis by exogenously administered PEG-rHuMGDF. In all 3 patients who had a response to PEG-rHuMGDF, MPV decreased as platelet counts increased, a finding similar to that in other clinical studies of PEG-rHuMGDF (data not shown).

In contrast to its potent activity with respect to platelet production, PEG-rHuMGDF had a minimal effect on red and white blood cells (data not shown).

Before the start of PEG-rHuMGDF therapy, Patients 1, 2, and 4 had bleeding episodes (ecchymoses and petechiae; Table 1). These decreased after administration of PEG-rHuMGDF, even in patient 1, whose platelet counts remained low.

In addition to the striking rise in platelet counts, there was a significant increase in the percentage of reticulated platelets in all patients except patient 2, in whom measurement of this variable was not performed (Figure 1). In patients 3 and 4, the increase in reticulated platelets reached the peak value on day 3 and day 6, respectively, preceding the platelet peak by 8 to 12 days. Even in patient 1, who had no platelet response, the percentage of reticulated platelets increased transiently and peaked on day 10, which might have been related to the decrease of bleeding episodes. The data on the reticulated platelets indicate that PEG-rHuMGDF therapy increased production of new platelets in these patients.

It was reported previously that neutralizing antibodies with consequent thrombocytopenia developed in approximately 4%
of healthy volunteers who received more than one dose of PEG-rHuMGDF. In the current study, however, no antibodies were detected in serum from any patient 6 months after the start of treatment.

Although data from only 4 patients are available, our current results strongly suggest that PEG-rHuMGDF may be clinically useful in patients with chronic ITP. PEG-rHuMGDF therapy might be effective in ameliorating thrombocytopenia and improving bleeding symptoms in some patients with chronic ITP refractory to standard therapy. For example, administration of PEG-rHuMGDF could be used to increase platelet counts transiently before elective surgery, such as splenectomy, in patients with ITP. The current dose-escalation study was discontinued at a dose of 0.5 μg/kg per day because the lowest dose of PEG-rHuMGDF used for 7 successive days caused an excessive increase in platelet counts in 2 of the 4 patients. Further studies must be conducted to determine the optimal dose and schedule of PEG-rHuMGDF administration in patients with chronic ITP.

Acknowledgments
We thank Drs Kiyoko Watanabe and Yohko Kawai for measurement of reticulated platelets.

References

Effects of pegylated recombinant human megakaryocyte growth and development factor in patients with idiopathic thrombocytopenic purpura

Shosaku Nomura, Kazuo Dan, Tomomitsu Hotta, Kingo Fujimura and Yasuo Ikeda