Megakaryocyte Growth and Development Factor Ameliorates Carboplatin-Induced Thrombocytopenia in Mice

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Megakaryocyte growth and development factor (MGDF) administered intraperitoneally (IP) to mice causes a dose-dependent thrombocytopenia accompanied by a decrease in mean platelet volume. MGDF increases the number of megakaryocytes in the bone marrow and spleen. MGDF does not affect the circulating number of leukocytes. Carboplatin, a chemotherapeutic agent that causes thrombocytopenia in humans, administered to mice as a single IP injection at a nonlethal dose causes a significant, but reversible thrombocytopenia. The carboplatin-induced thrombocytopenia is accompanied by an increase in circulating endogenous MGDF that precedes the return of circulating platelets to a normal level. MGDF mRNA is constitutively present in the liver. After carboplatin treatment, hepatic MGDF mRNA does not increase in concordance with circulating MGDF. Circulating soluble MGDF receptor levels (c-mpl) do not change significantly during the course of carboplatin-induced thrombocytopenia. MGDF injected IP once daily beginning 1 day after injection of carboplatin reverses carboplatin-induced thrombocytopenia in a dose-dependent fashion. The normalization of circulating platelet numbers in carboplatin plus MGDF-treated mice is accompanied by a normalization of megakaryocyte numbers in the bone marrow. In conclusion, MGDF, by increasing the number of marrow megakaryocytes and circulating platelets is an effective therapy for carboplatin-induced thrombocytopenia in mice.

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M E G A K A R Y O C Y T E  G R O W T H and development factor (MGDF) is a megakaryocyte growth and development factor identified in aplastic canine plasma. Dog, mouse, and human MGDF cDNA clones have been isolated. MGDF binds to c-mpl affinity columns and the biologic effects of MGDF are inhibited by soluble c-mpl. The c-mpl ligand has been independently cloned by several groups of investigators who have documented the in vitro and in vivo thrombopoietic effects of this novel protein.

Carboplatin, an analog of cisplatin, is a second-generation platinum drug that is clinically effective in malignant tumors such as ovarian carcinoma, testicular carcinoma, and small cell carcinoma of the lung. Carboplatin’s mechanism of action appears to be related to its ability to cross-link DNA. Among carboplatin’s toxicities are myelosuppression and thrombocytopenia.

The purpose of the present study is to report the ability of MGDF to significantly ameliorate carboplatin-induced thrombocytopenia.

MATERIALS AND METHODS

Human recombinant MGDF was expressed in Escherichia coli as a truncated form of the molecule. Specific activity (1 × 10⁶ U/mg) was measured with a factor-dependent murine cell line transfected with the human MGDF receptor (mpl) gene. Cells were cultured with graded doses of MGDF for 72 hours and proliferation was measured with the Cell Titer 96 Assay Kit according to manufacturer’s instructions (Promega, Madison, WI). One unit is defined as the amount of material resulting in 50% maximal proliferation. MGDF was injected intraperitoneally (IP) in a carrier solution of 1% normal mouse serum dissolved in sterile saline. MGDF bioactivity in serum was measured by survival assays using interleukin-3 (IL-3) dependent murine 32D, clone 23 cells expressing murine mpl receptor. One thousand viable cells are incubated with serum samples from mice held in a restrainer. White blood cell count, platelet count, and mean platelet volume (MPV) were measured with a Sysmex cell counter (Toa Medical, Kobe, Japan). Serum was collected by centrifugation of clotted whole blood obtained via cardiac puncture. Immunohistochemistry for the identification of megakaryocytes was performed with rabbit anti-human von Willebrand factor (Dako Corp, Carpenteria, CA) on 10% neutral buffered formalin-fixed, paraffin-embedded decalcified femurs sectioned longitudinally. The number of megakaryocytes in marrow and spleen per 10 randomly counted 400 x magnification fields were counted.

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Days after Injection of MGDF

FIG 1. MGDF (n = 5 mice per group) causes a dose-response dependent increase in circulating platelets (A) and a decrease in MPV (B) without any accompanying changes in circulating numbers of leukocytes (C).

using light microscopy by an observer blinded to the experimental groups. Statistics are expressed as the mean ± 1 SD.

RESULTS

MGDF injected IP to Balb/c mice causes a dose-dependent thrombocytosis (Fig 1A) accompanied by a decrease in mean platelet volume (Fig 1B). MGDF does not affect the circulating number of leukocytes (Fig 1C). MGDF does not affect the hemoglobin concentration in whole blood (data not shown). A single injection of 5 mg/kg MGDF causes an increase in circulating platelets beginning after 3 days and peaking after 5 days, providing an indication of the time elapsing between the initial stimulation of megakaryocyte...
Fig 3. Carboplatin (125 mg/kg) causes a rapid decrease in circulating platelet number between days 5 to 8 followed by a sustained thrombocytopenia and then rapid recovery of platelet numbers between days 15 to 17. MPV gradually increases following the onset of thrombocytopenia and normalizes when the platelet count normalizes (n = 10 mice per group). Endogenous MGDF bioactivity is undetectable before the onset of thrombocytopenia and then increases rapidly as the platelet count drops. MGDF bioactivity is expressed in a semiquantitative cell-based bioassay as the number of cells per microwell with a mean peak of approximately 1,000 to 2,000 cells/well on days 8 through 11 and with less than 100 cells/well on days 0 to 5.

Precursors in the marrow and the release of platelets into the peripheral circulation (Fig 2).

Carboplatin injected to Balb/c mice as a single IP injection at a dose of 125 mg/kg causes a significant, but reversible, thrombocytopenia (Fig 3). Peripheral platelet numbers stay at preinjection levels for the first 5 days after injection of carboplatin and then drop rapidly over days 6 to 8. The thrombocytopenia plateaus between days 8 to 15. Platelet numbers rapidly return to a control level over the next 2 days. The carboplatin-induced thrombocytopenia is accompanied by an increase in circulating endogenous MGDF bioactivity that precedes the return of circulating platelets to a normal level (Fig 3). The mean platelet volume increases during the period of thrombocytopenia and then returns toward normal as the platelet number normalizes. MGDF mRNA levels in the liver do not increase in concordance with circulating MGDF levels (Fig 4A). MGDF mRNA expression in the livers of control and carboplatin-treated mice on day 8 were studied in a larger number of mice to look more carefully for any increase in steady-state MGDF mRNA expression (Fig 4B). A very slight increase in the average ratio of MGDF/GAPDH mRNA expression in carboplatin-treated mice was noted by PhosphorImager analysis (MGDF/GAPDH ratio is 0.95 ± 0.12 in controls and 1.17 ± 0.13 in carboplatin-treated mice, P < .05), but the increase in hepatic steady-state MGDF mRNA does not parallel the magnitude of the increase in circulating MGDF bioactivity. Circulating soluble c-mpl receptor levels measured between days 0 and 14 (n = 26 mice killed before and during the course of carboplatin-induced thrombocytopenia) range between 1.5 to 4.6 ng/mL and do not show any recognizable increase or decrease corresponding to changes in circulating numbers of platelets.

MGDF injected at doses of 10, 25, and 100 μg/kg IP once daily beginning 1 day after injection of carboplatin

Fig 4. The kinetics of MGDF mRNA expression are shown at selected days before, during, and after the peak of circulating MGDF bioactivity (A). MGDF mRNA expression (as related to expression of GAPDH) does not show an increase concordant with the increase in circulating MGDF. A very slight increase in the average ratio of MGDF/GAPDH mRNA expression in carboplatin-treated mice (B) was noted on day 8, but the increase in hepatic steady-state MGDF mRNA does not parallel the magnitude of the increase in circulating MGDF bioactivity.
8.1

Days after Injection

2-1-v

0 5 7 9 11 13 15

Days After Injection

Fig 5. MGDF (10, 25, and 100 μg/kg) injected on days 1 through 12 causes a dose-response dependent acceleration in the recovery of platelets after injection of carboplatin (A) (n = 5 mice in each group). In a second experiment MGDF (100 μg/kg) injected on days 1 through 15 completely abrogates carboplatin-induced thrombocytopenia (B) (n = 5 mice in each group). Note the return of the platelet number to control levels after the cessation of MGDF injections in mice treated with MGDF alone (B).

reverses carboplatin-induced thrombocytopenia in a dose-dependent fashion (Fig 5A). In a second set of experiments in which the carboplatin-induced thrombocytopenia was not as severe, MGDF at 100 μg/kg completely prevented thrombocytopenia (Fig 5B). In a third experiment, the normalization of circulating platelet numbers in carboplatin plus MGDF-treated mice on day 10 (Fig 6A) is shown to be accompanied by a normalization of megakaryocyte numbers in the bone marrow (Fig 6B) and spleen (Fig 6C). The normalization of megakaryocyte numbers in the femur by MGDF treatment is histologically apparent after immunostaining for von Willebrand factor (Fig 7). Carboplatin causes a slight transient decrease in hemoglobin that is not significantly ameliorated by MGDF (data not shown).

Fig 6. In a third experiment, mice treated with MGDF (100 μg/kg) on days 1 to 9 were killed at day 10 after injection of carboplatin to evaluate the bone marrow (n = 5 mice/group). MGDF abrogates the carboplatin-induced thrombocytopenia (A). MGDF abrogates the carboplatin-induced decrease in megakaryocytes in the femur (B). MGDF abrogates the carboplatin-induced decrease in megakaryocytes in the spleen (C).
DISCUSSION

An intensive scientific search to discover a humoral factor that will promote the growth and differentiation of megakaryocytes has culminated in the discovery of the c-mpl ligand MGDF. MGDF may be useful in a variety of thrombocytopenic states in which inadequate platelet production plays a key role. Patients who have received chemotherapy for treatment of malignancy may develop severe thrombocytopenia that is potentially life-threatening because of the risk of hemorrhage. Thrombocytopenia limits the ability of oncologists to escalate chemotherapy doses and may, thereby, limit the hope for remissions or cures in cancer patients. An agent to accelerate platelet recovery after chemotherapy in a fashion analogous to the way granulocyte colony-stimulating factor (G-CSF) promotes neutrophil recovery would be extremely useful. The present study demonstrates that MGDF fulfills the promise of ameliorating thrombocytopenia in a mouse model of carboplatin chemotherapy. Carboplatin, a commonly used agent in cancer chemotherapy, is a well-documented cause of thrombocytopenia in patients. In mice receiving carboplatin, the circulating level of endogenous MGDF was shown to increase at the time of rapidly decreasing platelet numbers, suggesting a role for endogenous MGDF in the regulation of circulating numbers of platelets. Interestingly and perhaps somewhat unexpectedly, MGDF mRNA expression in the liver (as related to expression of the housekeeping gene GAPDH) does not show an increase concordant with the increase noted in circulating MGDF, suggesting the possibility of either posttranscriptional regulation of MGDF gene expression or a role for circulating c-mpl levels in the regulation of MGDF bioactivity. Soluble circulating c-mpl levels, however, did not change appreciably at the time of increase in circulating MGDF bioactivity. The possibility also exists that platelet membrane c-mpl acts as a sink for free MGDF in a fashion similar to the possible role of neutrophil membrane G-CSF receptors in the regulation of free circulating G-CSF levels.

The in vivo effects on platelet numbers of several growth factors other than MGDF have been previously studied. IL-6, for example, has been reported to elevate the platelet count in carboplatin-pretreated rats. IL-6 is also reported to accelerate recovery from hematopoietic depression in mice by stimulating multilineage hematopoiensis. On the other hand, IL-6 apparently does not mediate the thrombopoietic response to acute thrombocytopenia and is not as specific a growth factor for platelets as is MGDF. IL-3 is another growth factor that is hematologically more pleiotropic than MGDF, but that may nevertheless exert beneficial...
effects on platelet numbers in patients. The combination of IL-3 and IL-6 modestly enhanced platelet recovery after 5-fluorouracil induced thrombocytopenia in mice. IL-1, IL-7, and IL-11 have all also shown potentially promising effects on platelets, although definitive clinical trials remain to be conducted. Clinical trials with MGDF will be necessary to determine the potential usefulness of MGDF, not only after chemotherapy, but also after bone marrow transplantation, as well as in other thrombocytopenic conditions.

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