Brief report

Short-term granulocyte colony-stimulating factor and erythropoietin treatment enhances hematopoiesis and survival in the mitomycin C–conditioned Fancc−/− mouse model, while long-term treatment is ineffective

Madeleine Carreau, Lili Liu, Olga I. Gan, Johann K. Hitzler, John E. Dick, and Manuel Buchwald

Transient treatment with cytokines appears to improve hematopoietic function in Fanconi anemia; however, the effectiveness or adverse effect of long-term treatment is not known. The mitomycin C–treated Fancc−/− mouse provides a valuable model to address long-term efficacy of such treatment. Fancc−/− mice injected with granulocyte colony-stimulating factor, erythropoietin, or both cytokines showed a delay in mitomycin C (MMC)–induced bone marrow (BM) failure compared to untreated mice. However, long-term cytokine exposure followed by MMC challenges did not protect mice from the reduction of peripheral blood counts or the number of early myeloid progenitors. These results suggest that cytokine treatment may be beneficial only in the short-term, while long-term treatment is not protective for BM aplasia.

Introduction

Fanconi anemia (FA) is a severe bone marrow (BM) failure syndrome transmitted through autosomal recessive inheritance. Somatic cell fusion studies resulted in the classification of FA patients into 8 complementation groups, each corresponding to a separate gene defect. Of these disease genes, six have been cloned, although no molecular function has been definitively attributed to any of the gene products. The clinical manifestation of FA is defined by a progressive BM failure and, in the majority of cases, a multitude of congenital malformations. In addition, FA patients are at an increased risk of developing myelodysplasia, acute myeloid leukemia (AML), and solid tumors later in life. The long-term curative treatment of the hematologic manifestation of the disease is BM or peripheral blood stem cell transplantation using a sibling HLA-matched donor. Alternatives to BM transplantation include the administration of androgens and hematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO), which may transiently improve peripheral blood counts. Because treatment of FA patients with cytokines have been done on small cohorts and measured short-term effects, the long-term efficacy of such treatment and its impact on the progression of FA to myelodysplasia and acute myeloid leukemia have not been determined. The FA group C knockout (Fancc−/−) mouse model provides a unique opportunity to assess the long-term effect of G-CSF and EPO treatment on mitomycin C (MMC)–induced BM failure. Our results show that G-CSF administration, alone or in combination with EPO, results in a delayed onset of MMC-induced BM failure in Fancc−/− mice, but that long-term therapy with both cytokines may increase the sensitivity of Fancc−/− mice to MMC.

Study design

Mice, MMC injections, and cytokine treatment

Mice, 5- to 6-month-old wild-type and Fancc knockout previously described, from a BALB/c genetic background were injected intraperitoneally with MMC (Roche Diagnostics, Laval, QC, Canada) at 0.3 mg/kg diluted in saline solution, a dose previously shown to induce progressive BM failure in Fancc−/− mice. Control mice were injected with equivalent volumes of saline. Human recombinant G-CSF (Filgrastim; Amgen, Mississauga, ON, Canada) and EPO (Janssen-Ortho, Toronto, ON, Canada), were diluted in saline and administered subcutaneously 3 times a week at a dose of 5 μg per mouse (160 μg/kg) of G-CSF and 10 U per mouse (300 U/kg) of EPO. The animal experiments were approved by the Animal Care Committee of the Hospital for Sick Children, Toronto, ON, Canada.

Hematological analysis

Peripheral blood counts including erythrocytes (RBC) and leukocytes (WBC) were analyzed from heparinized blood collected from the mouse tail vein using an automated cell counter (Coulter Counter Z1, Coulter Electronics, Mississauga, ON, Canada) as previously described. For hematopoietic colony-forming cell (CFC) assay, BM cells were seeded in complete methylcellulose medium as previously described. Statistical analysis was performed using either the Student paired t test or a 1-way analysis of variance (ANOVA) statistical program.

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Results and discussion

Effect of cytokine treatment on MMC-induced BM failure in Fancc<sup>-/-</sup> mice

Although Fancc<sup>-/-</sup> mice have only subtle defects in their peripheral hematopoietic system without spontaneous BM failure, they have a significant stem cell defect. The mice are exquisitely sensitive to the DNA cross-linking agent MMC, which, when used in low doses, induces severe BM aplasia. Thus, the MMC-treated Fancc<sup>-/-</sup> mice are useful models that reflect the BM aplasia seen in FA patients. To determine if G-CSF therapy protects against BM aplasia in this model, we treated Fancc<sup>+/+</sup> and Fancc<sup>-/-</sup> mice with weekly injections of 0.3 mg/kg MMC, a dose known to induce progressive BM failure in Fancc<sup>-/-</sup> mice in combination with G-CSF, EPO, or G-CSF plus EPO. The survival, RBC and WBC counts were monitored weekly during the course of the experiment. Treatment with G-CSF, EPO, and the combination of cytokines significantly delayed the reduction of both RBC and WBC counts in MMC-treated Fancc<sup>-/-</sup> mice (Figure 1). However, cytokine treatment did not reverse BM aplasia, indicating that primitive stem cells were not affected by this treatment. Indeed, neither G-CSF nor EPO act on murine repopulating stem cells. MMC treatment had no effect on survival of Fancc<sup>+/+</sup> mice and, as expected, cytokine administration increased both RBC and WBC counts in these control mice. As predicted from the peripheral blood cell counts, G-CSF and EPO increased the survival time of Fancc<sup>-/-</sup> mice by 1 week when compared with mice receiving MMC without cytokines (survival of 3 weeks). In addition, Fancc<sup>-/-</sup> mice receiving both cytokines survived twice as long as the controls receiving MMC without cytokines (6 weeks, Figure 1C). Histopathologic analysis showed BM aplasia in all Fancc<sup>-/-</sup> mice receiving MMC treatment; neither G-CSF and EPO alone nor a combination of both was able to prevent BM failure. Taken together, these results indicate that short-term administration of G-CSF and/or EPO significantly delays the onset of MMC-induced pancytopenia in the peripheral blood of Fancc<sup>-/-</sup> mice but does not reverse BM aplasia.

Effect of long-term exposure to G-CSF and EPO on survival and peripheral blood counts of Fancc<sup>-/-</sup> mice

We established a long-term cytokine treatment model to determine if increased duration of cytokine treatment could trigger primitive cells into action. Moreover, this experimental design provokes a more subtle neutropenia than the study reported in Figure 1, more closely mimicking FA patients who undergo cytokine treatment in response to neutropenia. Fancc<sup>-/-</sup> mice were subjected to a single
MMC injection to induce neutropenia and decrease BM cellularity, as previously shown.\textsuperscript{12} We started cytokine injections one week after the MMC treatment and monitored RBC and WBC counts weekly (Figure 2A,B). Mice receiving EPO with or without G-CSF showed an increase of their RBC after 3 weeks of treatment as opposed to mice receiving either G-CSF alone or no cytokines. The WBC count increased after 3 weeks of cytokine treatment above control values. After 18 weeks another dose of MMC was given. If the stem and progenitor cells had expanded or been stimulated by long-term cytokine action, we would expect the mice to survive this challenge. However, all cytokine-treated \textit{Fancc}\textsuperscript{−/−} mice showed a dramatic decrease in RBC counts following the MMC challenge as compared to untreated mice, whereas all mice, including cytokine-treated and untreated mice, showed a decrease in WBC counts. Mice receiving both G-CSF and EPO did not survive the MMC challenge, with 5 of 6 mice dying from pancytopenia within 1 week and the remaining mouse within 2 weeks of the challenge (Figure 2E). The surviving mice were killed 8 weeks after challenge to establish BM cultures and analyze their BM histopathology. A reduction of granulocyte macrophage colony-forming units (Figure 2D) was observed in all MMC-challenged mice regardless of cytokine administration, when compared to control animals not receiving MMC. The number of erythroid burst-forming units (Figure 2C) remained at subnormal level, in keeping with the RBC count in peripheral blood at that time.

Throughout the experiment, we did not observe increased numbers of myeloid cells or the presence of leukemic blasts in the peripheral blood of \textit{Fancc}\textsuperscript{−/−} mice receiving G-CSF and/or EPO. However, it should be noted that \textit{Fancc}\textsuperscript{−/−} mice treated or not treated with MMC do not appear to have a significant risk of developing myelodysplastic syndrome and/or AML. Our results demonstrate that G-CSF and EPO alone or in combination are efficacious in transiently increasing peripheral blood counts in FA. Long-term administration of either G-CSF or EPO may be partially beneficial, although the administration of both cytokines does not prevent BM failure, suggesting an inability to act on the long-term repopulating stem cells, which are the primary defect in \textit{Fancc} mice. Indeed, there is a possibility that cytokine treatment may even accelerate BM hypoplasia. Conceivably, the long-term administration of these factors may result in a depletion of the stem cell compartment, which, as we have previously shown, contains a reduced number of long-term repopulating stem cells with impaired function and self-renewal capacity.\textsuperscript{13,14}

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References

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