CASE REPORT

Methimazole Induced Total Myeloid Aplasia with Delayed Recovery Despite Granulocyte Colony Stimulating Factor (G-CSF): Marrow Progenitor Recovery Kinetics

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Abstract An eighteen-year-old female with Graves thyrotoxicosis presented with methimazole-induced agranulocytosis and total myeloid aplasia. The bone marrow at presentation showed complete absence of myeloid precursors and striking plasmacytosis. 16 days later, myeloid precursors were still absent morphologically; however bone marrow flow cytometry and cell culture detected an improvement in myelogenesis, which was soon followed by clinical recovery of agranulocytosis. Neutrophil recovery was delayed until day 22 after cessation of methimazole despite G-CSF use, consistent with a direct toxic effect on committed myeloid cells. Our findings suggest that cell culture and flow cytometric evaluation of bone marrow myeloid progenitors can be used as a guide to anticipate neutrophil recovery.

Keywords Methimazole · G-CSF · Total myeloid aplasia · Bone marrow cell culture · Flow cytometry

Introduction

Methimazole (MMI), one of the most widely used antithyroid medications, is well known to cause agranulocytosis, defined as an absolute neutrophil count (ANC) <

$500 \times 10^6/L$. MMI-induced agranulocytosis is reported to occur in ~1 % of patients taking doses >30 mg per day [1, 2].

We discuss the findings of bone marrow flow cytometry of a case of MMI-induced agranulocytosis and total myeloid aplasia in a patient with Graves thyrotoxicosis, along with a description of the bone marrow culture results. The myeloid suppression was successfully treated with discontinuation of the offending drug and use of granulocyte colony stimulating factor (G-CSF) in this patient.

Case Presentation

An eighteen-year-old biracial (African American and Caucasian) female with history of Graves disease for 8 years, on MMI since diagnosis presented to our institution with 2–3 days history of sore throat, fever, a new onset painful, erythematous swelling underneath her left eyelid, left sided abdominal pain and bloody watery diarrhea. She had been on 15 mg of daily MMI for 8 years, however due to symptomatic hyperthyroidism 2 months prior to presentation; the dose of MMI was increased to 40 mg daily. She was febrile and tachycardic on presentation and met the criteria for thyroid storm based on Burch-Wartofsky score [3]. The rest of her physical examination revealed diffuse abdominal pain and preseptal cellulitis of her left eye.

A complete blood count on presentation to our hospital showed a white blood cell count of $900/\mu L$ with an ANC: 0 cells/ μL , absolute lymphocyte count: 600 cells/ μL , absolute monocyte count: 0 cells/ μL , hemoglobin: 10.7 g/dL, platelet count: $327,000/\mu L$, mean corpuscular volume: 66.3 fL, and an absolute reticulocyte count of $55,400/\mu L$. Further work up of her anemia revealed a normal hemoglobin evaluation thus ruling out thalassemia trait, negative

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Fig. 1 Changes in the blood absolute neutrophil count (a); reticulocyte and platelet counts (b). All counts are per μL . Day 0 is the day the last dose of MMI was taken. *P* denotes plasmapheresis sessions

coombs test, iron studies showed low iron: $25 \mu\text{g/dL}$ ($50\text{--}70 \mu\text{g/dL}$), low TIBC (total iron binding capacity): $199 \mu\text{g/dL}$ ($250\text{--}450 \mu\text{g/dL}$), low iron saturation: 13% ($20\text{--}50 \%$), and high ferritin: 333 ng/mL ($10\text{--}291 \text{ ng/mL}$) consistent with a picture of anemia of acute inflammation. Thyroid studies were as follows: Free T4: 10.7 ($0.8\text{--}1.8 \text{ ng/dL}$), TSH: <0.008 micro IU/mL, thyroid stimulating immunoglobulin: 403% (normal: $<123 \%$) and positive thyroid microsomal antibodies. There was no laboratory evidence of acute infection with Cytomegalovirus, Epstein Barr Virus, Human Immunodeficiency Virus 1 and 2, Human Herpesvirus 6, or Parvovirus B19. Anti-Granulocyte Antibody was negative.

She was started on intravenous Cefepime for empiric treatment of febrile neutropenia. Propranolol was started for the management of hypertension and tachycardia on the day of admission. MMI was discontinued, and the patient was started on G-CSF as a single dose of $10 \mu\text{g/kg/day}$ for the initial 15 days and later increased to $10 \mu\text{g/kg/day}$ twice daily for a total of 7 days (Fig. 1a). The thyroid storm was treated with dexamethasone, one dose of intravenous immunoglobulin (day 13), four rounds of plasmapheresis (days 16, 18, 21, and 23), cholestyramine resin, and surgical thyroidectomy 31 days after the last dose of the MMI. The patient's course was complicated by a) candidemia (day 10), treated with intravenous Amphotericin B; b) cerebral sinus venous thrombosis (day 10), treated with low molecular weight heparin. The patient

developed thrombocytopenia (day 12) and reticulocytopenia (day 18) in addition to neutropenia (Fig. 1b).

The reticulocyte count dropped to as low as $26,000/\mu\text{L}$ and the platelet count dropped to as low as $28,000/\mu\text{L}$. However, the counts slowly started to recover, with neutrophils rising to $>500/\mu\text{L}$ and reticulocytes $>50,000/\mu\text{L}$ on day 22 after the last dose of MMI. The platelet counts normalized only 43 days after the last dose of MMI. Figure 1a demonstrates the changes in absolute neutrophil counts along with the other clinical events. Figure 1b illustrates the changes in the reticulocyte and platelet counts.

Methods

The bone marrow mononuclear cells were cultured in semi-solid methylcellulose growth medium containing recombinant cytokines and erythropoietin (MethoCult H4435 Enriched; Stem Cell Technologies; Vancouver, CAN) supplemented with and without 10% patient serum before and after treatment with G-CSF. The colony assays were plated at 10^5 cells/well in triplicate and Colony Forming Units—Granulocyte, Macrophage (CFU-GM) and Burst Forming Units—Erythrocytes (BFU-E) colonies were scored on day 14. Paired *t* test was used to compare colony growth between the first and second bone marrow specimens.

Fig. 2 Photomicrographs of bone marrow aspiration (**a**) in original magnification ($\times 40$) showing myeloid aplasia and presence of plasma cells, (**b**) in original magnification ($\times 100$) showing increased number of plasma cells prior to starting G-CSF treatment

Results

Bone Marrow Colony Studies

The first bone marrow aspirate was obtained on the day MMI was discontinued, prior to initiation of G-CSF, and was mildly hypocellular with near complete absence of myeloid precursors, 39 % erythroid precursors, 33 % lymphocytes, adequate megakaryocytes, and striking plasmacytosis (28 % plasma cells) (Fig. 2a, b). The bone marrow myeloid-to-erythroid (M:E) ratio was 0. Flow cytometric analysis of bone marrow prior to G-CSF treatment showed only 4 % CD45 dim+ progenitors, with additional staining for myeloid progenitor specific markers CD33, CD34 and CD117 in the CD45 dim+ gated region showing presence between 0.04 and 0.13 % level of detection of total mononuclear cells (Fig. 3a–d). Bone marrow culture studies also showed low numbers of CFU-GM and BFU-E colonies.

The second bone marrow aspirate on day 16 after discontinuation of MMI, nearly 2 weeks after treatment with G-CSF, continued to show near absence of myeloid precursors and still high numbers of plasma cells but; this time decreased to 14 %. A bone marrow biopsy was done revealed hypocellular marrow (approximately 20 %) with decreased trilineage hematopoiesis. There was also dyserythropoiesis and around 15–50 % of plasma cells were noted. Flow cytometric analysis at this time, however, showed 26 % CD45 dim+ progenitor cells with additional CD33, CD34 and CD117 positive staining displaying significantly elevated myeloid progenitor cells in the 6.20–10.08 % level of detection of total mononuclear cells (Fig. 3e–h). Bone marrow culture studies also displayed a marked increase in the mean number of colonies in the wells containing CFU-GM and BFU-E colonies, with or without autologous serum treatment. In serum-treated

cultures, CFU-GM increased from 1.7 ± 0.6 colonies (mean $\pm$ standard deviation) to 85.0 ± 8.0 colonies ($p = 0.003$); and BFU-E increased from 1.0 ± 0 colonies to 22.0 ± 4.4 colonies ($p = 0.014$).

Discussion

Agranulocytosis due to antithyroid drug medications is a well-recognized serious side effect found to occur in ~ 1 % of patients [1, 4–7]. Two possible mechanisms for the pathogenesis of the MMI-induced agranulocytosis have been suggested: either the drug could directly show its toxic effects on the myeloid progenitors, and mature circulating neutrophils [5, 8, 9], or the drug could induce an autoimmune reaction targeting the neutrophils [10–13]. The findings in our patient suggest a direct toxic effect on hematopoietic progenitors because: 1—the patient developed total myeloid aplasia along with a decrease in other blood counts after 8 years on the drug, only after elevating the dose to 40 mg daily; 2—the culture studies from the second bone marrow 16 days after discontinuation of the MMI showed recovery of myeloid progenitor colonies even when autologous serum was added, thus negating potential antibody mediated suppression of the marrow [13]. Plasmapheresis was helpful in relieving the patient's thyroid storm, but possibly was of little benefit to neutropenia. We know from the bone marrow culture studies that the marrow was already recovering prior to implementation of the plasmapheresis.

We observed a delayed recovery of neutrophils despite the use of G-CSF consistent with direct toxicity to hematopoietic stem cells, in particular committed myeloid stem cells. In prior non-randomized studies, G-CSF was thought to reduce recovery period of neutropenia in MMI-induced agranulocytosis [14]. Our patient's neutrophil

Fig. 3 **a–d** Bone marrow flow cytometry plots on day 1 bone marrow showing 4 % CD45 dim+ progenitors/Side Scatter (SS) (**a**), with additional staining for myeloid progenitor specific markers CD34/CD11b (**b**), CD33 (**c**) and CD117 (**d**) in the CD45 dim+ gated region showing decreased percentages of myeloid progenitor cells. **e–h** Bone

marrow flow cytometry plots on day 16 bone marrow showing 26 % CD45 dim+ progenitor cells (**e**) with additional staining for myeloid progenitor specific markers CD34/CD11b (**f**), CD33 (**g**) and CD117 (**h**) in the CD45 dim+ gated region displaying significantly elevated percentages of myeloid progenitor cells

recovery, however took three weeks, much longer than reported in the literature. On day 16 after cessation of MMI, the bone marrow continued to show near total myeloid aplasia but there was abundant CFU-GM colonies growth over the next several days. The lag time to response to G-CSF suggests degree of depletion of committed myeloid progenitors and indeed pluripotent stem cells. Consistent with this, prior observations have shown that growth factors are not effective in cases with severely low ANC (<100) [15]. Bone marrow recovery from agranulocytosis after G-CSF treatment can be delayed when pre-treatment bone marrow M:E ratio is <0.5 (mean, 9.8 days) compared to patients who had a M:E ratio ≥ 0.5 (mean, 2.2 days) [16]. We also know from observations on patients who underwent bone marrow transplantation for aplastic anemia prior to the clinical use of G-CSF that the natural neutrophil (ANC) recovery occurs at a median of 17 days (range: 5–37 days) [17].

Our patient developed anemia and thrombocytopenia in addition to the neutropenia. These last two cytopenias could also be secondary to MMI toxicity, as cases presenting with aplastic anemia have been reported [6, 18, 19];

however, the bone marrow examination in our case only showed mild hypocellularity with no fatty replacement. What is more, other clinical complications could partly account for both the anemia and thrombocytopenia. The patient's iron studies were consistent with a picture of anemia of inflammation. Candidemia and cranial sinus venous thrombosis both could contribute to the thrombocytopenia. We suspect that thyrotoxicosis, by elevating coagulation factors and fibrinolysis inhibitors, itself may have induced the development of the thrombotic complication in our patient [20–22].

In conclusion, we describe a case of MMI-induced total myeloid aplasia with ANC: 0 and delayed recovery of bone marrow despite treatment with G-CSF. Prompt withdrawal of the offending drug is the most important step in the management. It is important to recognize that despite G-CSF, count recovery may be delayed up to 21 days or more. There should be a high index of suspicion of agranulocytosis for patients who develop fever or sore throat while on MMI. Such patients should be quickly assessed for an imminent sepsis and should receive appropriate treatment for febrile neutropenia.

Bone marrow examination and culture studies can be very helpful in the initial evaluation of a patient with MMI-induced agranulocytosis [23]. In our case, the follow-up bone marrow study was especially helpful because we could document an increase in myeloid precursors by flow cytometry and colony studies, although morphologically this was not immediately evident. Flow cytometric evaluation of the bone marrow can be used as a guide to anticipate neutrophil recovery in MMI-induced agranulocytosis and total myeloid aplasia.

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