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Case Report Open Access

Three Cases of Refractory Anemia with Ringed Sideroblasts Who Responded to Chloroquine

Mohammed Salhab[1], **Ali Zaidi**[2], **Iffat Adeel**[2], **Sana Saeed**[2] , **Zeba Aziz**[2], **Azra Raza**[2*] **and Michael Auerbach**[3]

[1]*Brown University Medical Center, USA*

[2]*Columbia University Medical Center, USA*

[3]*Georgetown University Medical Center, USA*

[*]**Corresponding author**: Azra Raza, Columbia University Medical Center, New York, USA, Tel: 2123058291; 6468086471; Fax: 2123056891; E-mail: azra.raza@columbia.edu

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Abstract

We report three cases of myelodysplastic syndrome (MDS), refractory anemia with ringed sideroblasts (RARS), who responded to the anti-malarial chloroquine in combination with recombinant erythropoietin (darbepoetin alfa). All three patients either remained or achieved transfusion independence and increased their hemoglobin level by at least 1 gm/dl. Responses occurred rapidly, becoming clinically noticeable within the first 4-6 weeks from start of therapy. All patients were followed for at least a 6 months period during which they have remained transfusion independent. Chloroquine affects multiple cellular pathways, but the precise mechanism of its action in RARS patients is unclear.

Keywords: Myelodysplastic syndromes; Refractory Anemia with Ringed Sideroblasts (RARS); Chloroquine; Autophagy

Abbreviations:

MDS: Myelodysplastic Syndromes; RARS: Refractory Anemia with Ringed Sideroblasts; Hgb: Hemoglobin; g/dl: Gram/deciliter; WBC: White Blood Count; ANC: Absolute Neutrophil Count

Introduction

Refractory anemia with ringed sideroblasts (RARS) is a common, low grade subtype of myelodysplastic syndromes (MDS) characterized by the presence of erythroblasts showing iron filled mitochondria around the nucleus [1]. Two distinct types of RARS are recognized on the basis of finding refractory anemia with uni-lineage dysplasia (RARS) or refractory cytopenias with multi-lineage dysplasia and ringed sideroblasts (RCMD-RS). The clinical outcome is better for the former [1]. For the purpose of this review, both will be referred to as RARS. The molecular events responsible for the RARS phenotype were unclear until 2011 when two groups independently performed whole exome sequencing in MDS patients and identified somatic mutations in the RNA splicing factor gene SF3B1 in 60-80% patients with RARS [2]. Mutations were heterozygous substitutions clustered in exons 12-16 with a hot-spot K700E accounting for ~50% of the observed variants. RARS patients tend to have a chronic, slowly progressive course with relatively long survival. Anemia is their major problem with most patients eventually becoming transfusion dependent and requiring treatment. Allogeneic hematopoietic stem cell transplant (HSCT) is the only potentially curative option but current guidelines recommend supportive care followed by a trial of disease modifying agents with HSCT held in abeyance until disease progression [3]. Palliative treatment options include growth factors, lenalidomide, hypomethylating agents (HMA) and experimental trials. Erythropoiesis stimulating agents (ESA) are less effective for the anemia of RARS [3] but responses improve when combined with granulocyte colony stimulating factor (G-CSF). Lenalidomide is approved for when del (5q) is present and HMAs for the rest. Responses to HMA after ESA resistance are in the range of 17% and even among the responders, the duration of response is ~44 weeks. In summary, for the majority of RARS patients, even if responses are achieved, they generally do not last very long and eventually all revert to transfusion dependency [1,3]. New treatment strategies are required which might be utilized sequentially in these chronically anemic patients.

In this report, we present three cases of RARS who were refractory to ESA but responded to the addition of the anti-malarial drug chloroquine by remaining or becoming transfusion independent and increasing their Hb by at least 1 gm in each case. Potential benefits and risks, especially the gastrointestinal and ophthalmic complications, were explained in detail to the patients who had close follow up for the first 8 weeks until counts improved and each was subjected to a quarterly ophthalmic examination.

Case 1

A 48-year-old man with past medical history of Gilbert syndrome was diagnosed with macrocytic anemia. There were no symptoms and the physical exam was normal. There was no history of alcohol or toxic exposure. A complete blood count (CBC) revealed hemoglobin (Hgb) of 13 g/dl with normal white blood cell (WBC) and platelet counts. The peripheral blood smear was normal as were the renal and liver function tests, levels of vitamin B12, folate, serum iron, ferritin, percent transferrin saturation and LDH. An intrinsic factor antibody assay was negative. Over the next year the Hgb decreased to 11 g/dl. A bone marrow aspirate and biopsy revealed a hypercellular bone marrow (30-50%) with prominent erythroid precursor cells. Occasional dysplastic erythroid cells without abnormalities in granulocyte cells or in the megakaryocytes were noted. Greater than

15% ringed sideroblasts, with 3+ marrow iron on Prussian blue stain was reported. No increase in blasts (less than 5%) was present. Cytogenetic analysis revealed a normal male karyotype (46, XY). No cytogenetic abnormalities were detected in FISH analysis with probes for EGR1 (5q-), D7S486 (7q-), CEP8 (trisomy8), and D20S108 (20q-). No diagnostic immunophenotypic abnormalities were detected on flow cytometry, darbepoetin alfa 200 mcg every two weeks was started. Dose escalations with addition of pyridoxine resulted in no improvement. Two years later mild neutropenia (ANC 1100/microliter) and a drop in Hgb to 9.5 g/dl were noted. Increased fatigue with a normal physical exam was noted. The peripheral blood smear revealed hypolobation, hypogranulation and pelgerization of neutrophils. A repeat bone marrow showed increased cellularity (75%), numerous ring siderblasts and no increase in blasts, darbepoetin alfa dose was increased to 400 mcg every two weeks with G-CSF and WBC improved but the Hgb decreased to 6.8 g/dl. Two units of packed red blood cells were administered with marginal clinical benefit. Chloroquine 250 mg daily was added to darbepoetin alfa 400 mcg every 4 weeks. The dose of chloroquine was increased to 500 mg daily at three weeks. At 5 weeks, a one gram increment in the Hgb was observed (7.6 g/dl). Six months later the Hgb increased to between 8.5-9 g/dl. WBC and platelets counts remained normal. No blood transfusions were administered, darbepoetin alfa doses were spaced to every six weeks. An improved level of energy and performance was reported. Ophthalmologic exams remained unchanged. Mild gastrointestinal disturbances were present during the first two months of therapy and abated thereafter (Figure 1).

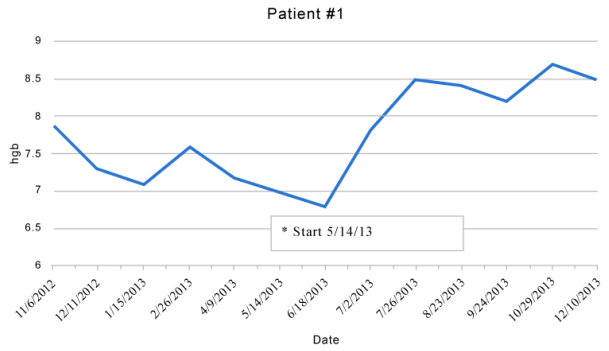

Figure 1: Increase in Hb in response to the addition of the anti-malarial drug chloroquine by remaining or becoming transfusion independent.

Case 2

A 78-year-old woman presented with a macrocytic anemia along with a past medical history of hypertension, stage 3 chronic kidney diseases and hemochromatosis (double heterozygote) for which regular phlebotomies were performed. Other than mild fatigue there were no symptoms. A physical exam was normal. Initial laboratory workup revealed an Hgb of 11.7 g/dl and MCV 102. The creatinine clearance was 40 milliliter per minute, serum ferritin 444 µg/L and percent transferrin saturation of 41. There was no history of alcohol or toxic exposure, darbepoetin alfa 200 mcg ever four weeks was started and increased to 400 mcg every 4 weeks over the next several months, in an attempt to keep the Hgb high enough for regular phlebotomies every 12-16 weeks. One year later a drop in Hgb to 8.6 g/dl occurred. Increased fatigue was reported. The MCV was 113 with normal WBCs, platelets, folate, vitamin B12 and LDH levels. Peripheral blood smear revealed hypolobation, hypogranulation and pelgerization of neutrophils. A bone marrow aspirate and biopsy revealed a hypercellular bone marrow with marked hyperplasia of erythroid series with >15% ringed sideroblasts, and +3 iron on Prussian blue stain. No increase in blasts (less than 5%) was seen. Cytogenetic analysis revealed a normal female karyotype (46, XX). No cytogenetic abnormalities were detected in FISH analysis with probes for EGR1 (5q-), D7S486 (7q-), CEP8 (trisomy8), and D20S108 (20q-). No immunophenotypic evidence of a hematolymphoid neoplasm on flow cytometry was observed. Pyridoxine was added at 250 mg per day but an increment to 500 mg per day as a single or divided dose was not tolerated due to gastrointestinal symptoms. Phlebotomies were discontinued. The iron parameters remained in a safe range obviating the need for iron chelating agents. The Hgb slowly decreased over the next two years to a nadir of 7.6 g/dl with persistent fatigability. No transfusions were administered. Chloroquine 500 mg daily was added to darbepoetin alfa. Nausea and headaches occurred two weeks after starting chloroquine. A dose decrement to one gram weekly was subsequently tolerated. At 4 weeks the Hgb increased by 1 g/dl. The WBC and platelet counts were stable throughout. Six months later the Hgb remained stable at approximately 9 g/dl. No transfusions were needed. The energy level improved with less fatigue, darbepoetin alfa doses were extended to every 4 weeks (Figure 2).

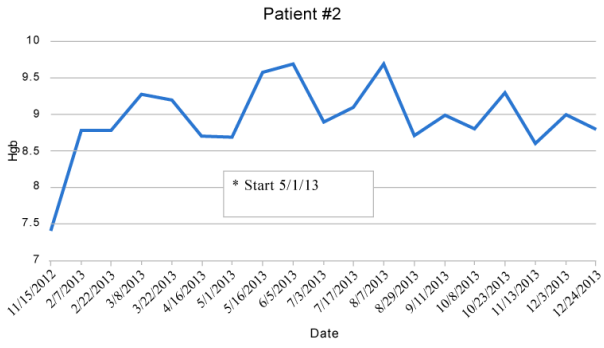

Figure 2: No immunophenotypic evidence of a hematolymphoid neoplasm on flow cytometry was observed

Case 3

A 72-year-old male with a past medical history of hypertension, coronary artery disease and stage 4 chronic kidney diseases was referred for unexplained macrocytic anemia. There were no symptoms other than fatigue. The physical exam was normal except for a soft systolic murmur at left lower sternal border. A midline thoracic scar was noted. There was no history of alcohol or toxic exposure. Initial blood work revealed an Hgb of 8.2 g/dl with an MCV of 110. Normal levels of iron, ferritin, folate, vitamin B12 and LDH were noted. A peripheral blood smear revealed round macrocytes, circulating normoblasts, dysmorphic erythrocytes and teardrops. A bone marrow aspirate and biopsy revealed hypercellularity (90%) with trilineage hematopoiesis including profound left-shifted erythroid hyperplasia with dyserythropoiesis and granulocytic hypoplasia without increased blasts (less than 1%), greater than 50% ringed sideroblast, with +3

amounts of iron on Prussian blue stain. Cytogenetic analysis revealed a normal male karyotype (46, XY). No cytogenetic abnormalities were detected by FISH analysis. No immunophenotypic evidence of a hematolymphoid neoplasm on flow cytometry was observed; darbepoetin alfa 200 mcg every two weeks was started and titrated up to 400 ug. Pyridoxine was added with transient improvement in Hgb. One year later the Hgb fell to 7.6 g/dl with increased fatigue. Chloroquine 500 mg daily was added to the darbepoetin alfa. The Hgb increased steadily. The dose was decreased to 375 mg daily because of dyspepsia. Three months later (April 2013), patient was admitted due to abdominal pain and stopped taking chloroquine. The hemoglobin fell to 7.2 grams. After discharge the chloroquin was restarted at 250 mg per day. The Hgb increased by 1 gm/dl in two months and then stabilized. Seven months later an ophthalmologist found unusual corneal changes with no retinal changes. The chloroquine was stopped and the Hgb decreased to 8 g/dl in less than 4 weeks. The ophthalmologist recommended restarting cholroquine at 250 mg daily. The Hgb increased, to 8.7 gm/dl and has remained stable. No transfusions have been administered and the darbepoetin alfa doses were spaced further apart. An increased energy level with improved performance continues. Patient was followed up most recently in March 2014 and remains transfusion independent. The hemoglobin on 2750 mg chloroquine per week was 8.2 g/dl, two grams higher than it was on darbeopoietin alone. The white count is 700/mm3 higher and the platelet count unchanged. This sustained response to chloroquine is impressive (Figure 3).

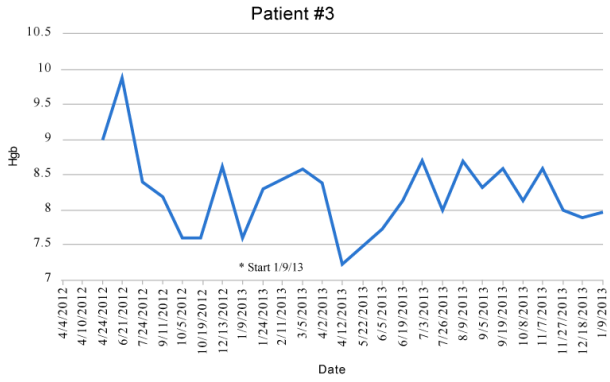

Figure 3: No transfusions have been administered and the darbepoetin alfa doses were spaced further apart

Discussion

Three consecutive RARS patients treated with chloroquine and darbepoetin alfa responded. Responses were observed in the first 4-6 weeks of therapy, and the Hgb continued to improve or stabilize. All three had initially been treated with darbepoetin alfa for at least 8 weeks without response. Typically RARS is resistant to ESAs unless given in combination with G-CSF. All three have been followed for at least 6 months and remain transfusion independent. Chloroquine, an old anti-malarial agent, has previously been used for the treatment of many cancers including melanoma, glioblastoma, lung cancer, breast cancer and RARS [4-7]. Following the initial report of a complete response to chloroquine in an RARS patient [7], and its success in porphyria cutanea tarda [8], suggesting interferences with heme synthesis, chloroquine has been tried in RARS sporadically, but these attempts did not meet with consistent success [9]. We reverted to this rather benign, non-invasive therapeutic approach and have been encouraged by achievement of transfusion independence and/or increase in Hgb by at least 1 gram in 3/3 consecutively treated patients with low risk RARS.

The precise mechanism of its action in cancer cells remains unknown, but chloroquine affects a broad range of cellular signaling pathways. The following possibilities can be considered:

Inhibition of autophagy, a mechanism which is noted to be hyper-efficient in malignant cells. Autophagy is responsible for the degradation of intracellular organelles via the lysosomal machinery thereby helping to maintain a balance between synthesis-degradation and recycling of cellular products. Inhibition of autophagy is being tried as a novel strategy for cancer therapy [10,11].

Induction of p53-mediated apoptosis is one of the mechanisms underlying the growth-suppressing effects of chloroquine in glioma cells and could also be an important mechanism of its action in MDS [12].

Chloroquine promotes intracellular Hgb accumulation and limits intracellular Hgb clearance which could be contributing to the increase in Hgb observed in our cases [13].

Chloroquine has been used in many autoimmune diseases because of its immunomodulatory functions, and this could be another mechanism by which the drug is acting [14].

In conclusion, we present 3 consecutive cases of RARS which achieved complete transfusion independence or increase in Hgb in response to chloroquine. All three received concomitant darbepoetin alfa which was previously ineffective as a single agent. The mechanism of action of chloroquine likely involves a combination of its effects on autophagy, induction of apoptosis in MDS cells through upregulation of p53, intracellular retention of Hgb and a more general immunemodulatory function. The specificity of response to chloroquine in RARS patients suggests a mitochondrial related mechanism of action and could involve attenuation of mitophagy [15]. Larger controlled trials are warranted to test this therapeutic strategy either as a single agent or in combination with other drugs known to be effective in MDS.

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