Hydroxyurea Increases Hemoglobin F Levels and Improves the Effectiveness of Erythropoiesis in β-Thalassemia/Hemoglobin E Disease

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Hydroxyurea (HU) is one of several agents that have been shown to enhance hemoglobin (Hb) F levels in patients with sickle cell disease and may be useful as a therapy for β-globinopathies. However, limited information exists on the effects of HU in patients with thalassemia. Accordingly, we examined the hematologic effects of orally administered HU in 13 patients with β-thalassemia/Hb E, including four patients who had been splenectomized. These patients were treated with escalating doses (final range, 10 to 20 mg/kg/d) for 5 months and were observed in the outpatient hematology clinic every 2 to 4 weeks. Complete blood counts including reticulocyte counts, amounts of Hb E and Hb F, γ:α and α:non-α globin biosynthetic ratios were evaluated before and during treatment. Almost all patients responded with an average increase of 33% in Hb F levels, from a mean (±SD) of 42% ± 11% to 56% ± 8% (P < .0001), and a reciprocal decline in the percentage of Hb E from 59% ± 9% to 49% ± 8% (P < .001). Reticulocytosis was decreased from a mean (±SD) of 18.0% ± 15.6% to 11.7% ± 9.1% (P < .05); there was also a slight (10%) but statistically significant increase in hemoglobin levels and an improved balance in α:non-α globin chains ratios. The side effects were minimal in most patients, although these patients tended to tolerate a lower dose of HU before significant myelosuppression than has been our previous experience in sickle cell disease. One spleenectomized patient died of sepsis during the trial. We conclude that increased Hb F production in β-thalassemia/Hb E patients, with an improvement in the α:non-α globin ratios and, probably, the effectiveness of erythropoiesis, can be achieved using HU. Longer trials of HU in this population, including at other doses and in combination with other agents, appear warranted.

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THALASSEMAIA is a heterogeneous group of genetic defects that results in defective globin synthesis. It is the most common genetic disorder among the people living in Southeast Asia; α-thalassemia, β-thalassemia, hemoglobin (Hb) E, and Hb Constant Springs (CS) are prevalent. The gene frequencies of α-thalassemia reach 20% to 30% in Northern Thailand and Laos. Frequencies of β-thalassemia gene vary between 1% and 9%. Hb E is the hallmark of Southeast Asia, attaining a gene frequency of 50% to 60% at the junction of Thailand, Laos, and Cambodia. Hb CS frequencies vary between 1% and 8%. These abnormal genes in different combinations lead to 60 thalassemia syndromes. Interaction between β-thalassemia and Hb E genes leads to homozygous β-thalassemia and β-thalassemia/Hb E diseases that are major β-thalassemia syndromes in this region. β-thalassemia/HbE can be as severe as homozygous β-thalassemia. Indeed, hemoglobin levels in 802 cases of β-thalassemia/Hb E disease at steady state varied from 2.6 to 13.3 g/dL, with an average of 7.7 g/dL.

Many factors may contribute to this heterogeneity, such as concomitant inheritance of α-thalassemia, which can ameliorate the severity of β-thalassemia. Recently, we found that the inheritance of a β-thalassemia chromosome with the Xmn I cleavage site at position -158 of the γ-globin gene that was linked to the haplotype -158- was associated with a milder anemia, as in the case of homozygous β-thalassemia. Increased expression of the γ-globin gene and higher production of hemoglobin F, which could reduce the overall globin chain imbalance, were also associated with homozygosity for the Xmn I cleavage site and, thus, with less severe anemia.

The finding that higher production of Hb F paralleled higher hemoglobin levels in β-thalassemia/Hb E patients was similar to findings in other β-thalassemia disease and sickle cell anemia. During the last 10 years, there have been several studies attempting to enhance Hb F production in patients with β-thalassemia and sickle cell anemia, which could be a therapeutic approach for these patients. Among these agents, hydroxyurea (HU), a cell cycle-specific agent that blocks DNA synthesis, has been shown to enhance Hb F levels in both nonhuman primates and in patients with myeloproliferative and sickle cell disease. Although early results in thalassemia have been variable, recently it has been reported that virtually all of 14 patients with sickle cell/β-thalassemia treated with HU have had an increase in Hb F levels and an apparent decrease in crisis frequencies. In this study, we examined the hematologic effects of orally administered HU in 13 patients with β-thalassemia/Hb E.

PATIENT SELECTION AND METHODS

Patients were considered eligible for the study if they exhibited signs of moderate to severe thalassemia, including evidence of osteoporosis.

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RESULTS

Fetal hemoglobin responses. Overall, patients responded with a 32.5% increase in fetal hemoglobin from a mean (±SD) of 42% ± 11% to 56% ± 8% (P < 0001). As indicated in Table 1, while there was a spectrum in the Hb F responses, only 1 of the 13 patients (patient 8) did not show a significant increase in Hb F expressed in grams per deciliter. In addition to the modest increase in the Hb F levels, most patients described an enhanced sense of well-being in comparison with their symptoms before treatment. Because this was not a controlled trial, we cannot formally exclude a placebo effect.

Clinical effects. In addition to the modest increase in the fetal hemoglobin, most patients described an increase in exercise tolerance and a feeling of well-being in comparison with their symptoms before treatment. Because this was not a controlled trial, we cannot formally exclude a placebo effect.

Side effects were minimal in most patients, except for the

enhanced production of fetal hemoglobin (Table 2). We were unable to detect an absolute relationship between the initial percent Hb F, $\alpha$-$\gamma$ ratio, and basal level of renal or hepatic function with the magnitude of the Hb F response. Fetal hemoglobin was detected in all of the red cells (F cells) before and during therapy by the modified Kleihauer acid-elution technique.21 Thus, we estimate that with treatment the proportion of Hb F per red cell increased by about one third.

Hematologic responses. There was a small but statistically significant increase in the level of hemoglobin, from 6.6 ± 0.6 g/dL to 7.3 ± 0.7 g/dL (Student’s t-test, P < 02). When a more conservative method of analysis (repeated measures—ANOVA with Bonferroni’s correction) was applied, taking into account the multiple measurements of hemoglobin before and at entry, this change in hemoglobin remained statistically significant (F = 6.95, P = .15; with Bonferroni correction, P < .05). There was also a moderate increase in bilirubin (4.9 ± 1.6 mg/dL to 3.5 ± 1.9 mg/dL, P < .001) and the reticulocyte count (18.0% ± 15.6% to 11.7% ± 9.1%, P < .05). As noted in previous clinical trials, HU therapy was associated with a marked increase in the mean red cell volume (MCV) and hemoglobin content (MCH), while there was no change in the mean corpuscular hemoglobin concentration, which is calculated from these two indices. Changes in these parameters correlated with the increments in fetal hemoglobin augmentation (r = .61, P = .03; Table 1). A summary of all patients’ hematologic profiles is shown in Table 2. Subset analysis indicated that there were no statistically significant differences before or after HU treatment in any of these listed parameters between the splenectomized and nonsplenectomized patients.

During this 20-week trial, there was indication of mild hematopoietic toxicity in the majority of our patients. The maximal average tolerable dose in these patients was 15 mg/kg/d (range, 11.1 to 17.9 mg/kg/d). There was a significant decline in the white blood cell (WBC) count (average decrease, approximately 50%) in 3 of 13 patients, in the platelet count (average decrease, approximately 30%) in 3 of 13 patients, and in the reticulocyte count in 1 of 13 patients. It should be noted that the nadir of these counts in most patients was at a level above what was considered significant hematologic suppression (see Patient Selection and Methods), and only three patients required a dose adjustment due to WBC count less than 3,000/μL. These declines in blood cell counts occurred precipitously within the 2 weeks between clinic visits and are to be distinguished from the more gradual decline in the WBC and reticulocyte counts occurring over the 5-month treatment period (Table 2). Thus, it may be concluded that requisite doses of HU to stimulate fetal hemoglobin levels in patients with β-thalassemia/Hb E are at or near the threshold for myelosuppression.

Clinical effects. In addition to the modest increase in the hemoglobin, most patients described an increase in exercise tolerance and a feeling of well-being in comparison with their symptoms before treatment. Because this was not a controlled trial, we cannot formally exclude a placebo effect.

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The results demonstrated that increased Hb F production in patients with β-thalassemia/Hb E associated with an improvement in the α/non-α globin production can be achieved using HU. Red cell MCV and MCH were also increased in all subjects (Table 2). Although there were no dramatic changes in the hemoglobin levels, the percentage and the absolute amount of reticulocytes were decreased, which reflects the improvement in bone marrow stress due to tissue anoxia. Alternatively, this decrease in reticulocyte count may reflect marrow toxicity. However, the fact that the reticulocytes decreased after the hemoglobin increased and that the patients exhibited a decrease in serum bilirubin and lactic dehydrogenase (LDH; data not shown) would support the improved erythropoiesis hypothesis. The temporal relationship between the increase in the percent HbF (and the reciprocal decrease in percent HbE) and the increase in total hemoglobin (Fig 2) is also consistent with this view. Bone marrow failure due to aplastic crisis (and presumably secondary to myelotoxicity) in the severe β-globin disorders may be accompanied by a transient decrease in the reticulocyte count and bilirubin and LDH levels, and a transient increase in Hb F levels. However, such effects are most often accompanied by more profound declines in the reticulocyte count (often to zero) and a concomitant decrease in hemoglobin levels, while the mean (red) cell volume remains unchanged — findings in marked contrast with our observations. Finally, we observed that the hemoglobin levels and red cell indices in these patients returned to baseline levels within 8 weeks of cessation of HU (data not shown), again
Fig 1. Effect of HU on hematologic values in a patient with β-thalassemia/Hb E. (A) Sequential change in hemoglobin values. (B) Reciprocal changes in the proportion of Hb F. Reflecting that the changes in hematologic parameters were coincident with HU therapy.

It is noteworthy that in these patients, the fetal hemoglobin and total hemoglobin levels continued to increase during treatment, and thus, the absolute improvement in the level of ineffective erythropoiesis and the effects on the characteristic peripheral hemolysis of these patients achievable with HU cannot be stated definitively. Indeed, long-term HU treatment of patients with sickle cell anemia indicates that a continued improvement in Hb F levels and in the hemoglobin values can be demonstrated for up to a year while on a stable dose of HU (G.P.R., unpublished observations, December 1994).

Fetal hemoglobin levels in patients with beta-globin disorders are determined by three factors: F-cell production, the amount of Hb F per F cell, and the preferential survival of F cells. We had previously shown that during short-term trials of HU in patients with sickle cell anemia, F-cell production, as estimated by the F-erythrocyte levels, accounted for 70% of the increase in Hb F levels. Unfortunately, it was not possible to study F-erythrocyte levels because of technical limitations in our method. The enumeration of F cells by acid-elution shows that all red cells in patients with β-thalassemia/Hb E give a positive reaction with heterochromatic staining for Hb F. Ideally, it would be necessary to use immunofluorescence staining and flow cytometry to distinguish increases in the F-cell production and/or the quantity of Hb F per F cell.

Fig 2. Effect of HU on hematologic values in 13 patients with β-thalassemia/Hb E. The averages and standard deviations in the percentages of (A) Hb F, (B) Hb E, and (C) total hemoglobin in this group of patients during HU treatment is shown.
Because of the short period of this clinical trial, it is very difficult to draw conclusions on the clinical response to HU treatment. Most of the patients reported feeling better; side effects generally have been minimal. In this group of patients who exhibit a β-thalassemia intermedia phenotype, even a modest increase in red cell production with coincident suppression of bone marrow turn-over might be highly beneficial; the need for transfusion support might be delayed or eliminated. Further studies will be required to evaluate the long-term toxicity and benefit to the patients. It is notable that these patients with β-thalassemia/HbE seem to tolerate a lower dose of HU than has been reported in patients with sickle cell anemia\(^1\)\(^2\) before hematopoietic toxicity is evident. Nonetheless, we have treated two Chinese patients with β-thalassemia intermedia for periods of 1 and 2 months before the initiation of HU treatment; *P* value determined by two-tailed Student’s *t* test. 

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