Original Article

Effects of Low Dose Hydroxyurea Treatment on Patients' Quality of Life in β-Thalassemia/Hemoglobin E Disease

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Abstract:

In clinical trials, treatment with hydroxyurea (HU) at a dosage of 20 mg/kg/day for 20 weeks has been shown to increase the production of fetal hemoglobin (Hb F) in patients with β-thalassemia/hemoglobin E disease (β-thal/HbE). The objective of the present study was to determine the hematological effects and toxicity of long term treatment with HU. Twenty patients with β-thal/HbE, including 6 nonsplenectomized and 14 splenectomized subjects, were treated with 10 mg/kg/day HU for 5 days/week for 36 months. Three patients were withdrawn from the study after 18 months, including two post-splenectomized patients who developed right-sided cardiac failure from chronic pulmonary thromboembolism and one who developed acute lymphoblastic leukemia. On average, HU treatment induced a 22.3% increase in absolute Hb F levels, with a reciprocal decline in Hb E levels. Hb levels, hematocrit levels and mean corpuscular volume (MCV) were slightly increased by HU treatment. Serum transferrin receptor levels were also significantly decreased by HU treatment, indicating some improvement in the degree of ineffective erythropoiesis. Reticulocytosis was decreased and a tendency towards an increase in the $^{G}\gamma/^{A}\gamma$ ratio was observed. The side effects of HU therapy were minimal and no evidence of myelosuppression was observed. All patients experienced an increase in the sense of well being and an improved quality of life. In conclusion, low-dose HU therapy administered five days weekly is well tolerated and effective for increasing Hb F levels and Hb levels in patients with β thalassemia/Hb E disease.

Keywords : ● *β- thalassemia* ● *Hydroxyurea* ● *Hemoglobin F-stimulation*

J Hematol Transfus Med 2017;27:151-8.

Received 27 February 2017 Accepted 15 March 2017

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บทคัดย่อ

วัตถุประสงค์งานวิจัยเพื่อ ศึกษาผลของการรักษาด้วย Hydroxyurea ในระยะยาวต่อลักษณะค่าต่างๆ ทางโลหิตวิทยาและผลข้างเคียงที่เกิดขึ้น ผู้ป่วยที่เป็นโรค β- thalassemia/Hemoglobin E disease (β-thal/HbE) จำนวน 20 คน ได้เข้าร่วมในงานวิจัย โดย 14 คนใน 20 คน เป็นผู้ป่วยที่เคยได้รับการผ่าตัดเอาม้ามออก และ 6 คนที่เหลือเป็นผู้ป่วยไม่ได้รับการผ่าตัดเอาม้ามออก ทั้งหมดได้รับการรักษาด้วย Hydroxyurea ขนาด 10 mg/kg/day เป็นเวลา 5 วันต่อสัปดาห์ นาน 36 เดือน ระหว่างทำการศึกษา 3 คนจาก 20 คน ได้ถอนตัวออกจากการศึกษาหลังทำการศึกษาได้ 18 เดือน โดย 2 ใน 3 เป็นผู้ป่วยที่อยู่ในกลุ่มที่เคยได้รับการผ่าตัดม้ามออกมาก่อน พบว่ามีภาวะหัวใจซีกขวาวาย เนื่องจาก โรคลิ่มเลือดอุดตันในหลอดเลือดปอดเรื้อรัง ส่วนอีก 1 คนที่เหลือ เกิดภาวะมะเร็งเม็ดเลือดขาว จากผลการรักษา พบว่า การรักษาด้วย Hydroxyurea มีผลเพิ่มระดับเม็ดเลือด HbF และลดระดับเม็ดเลือด HbE ส่วนระดับความเข้มข้นเลือด Hemoglobin, Hematocrit และค่า MCV เพิ่มขึ้นเล็กน้อยภายหลังการรักษาระดับ serum transferrin receptor ลดลงอย่างชัดเจนภายหลังการรักษา ซึ่งนั่นแสดงให้เห็นถึงภาวะ ineffective erythropoiesis ที่ดีขึ้นภาวะ Reticulocytosis ลดลง และสัดส่วน $^{G}\gamma/^{A}\gamma$ ratio มีแนวโน้มสูงขึ้น ส่วนผลข้างเคียงของการรักษาด้วย Hydroxyureaนั้น มีน้อยและไม่มีหลักฐานแสดงถึงการทำงานบกพร่องในการสร้างเม็ดเลือด ผลการรักษาทำให้ผู้ป่วย รู้สึกมีสุขภาพ และคุณภาพชีวิตที่ดีขึ้น โดยสรุปการให้ยา Hydroxyurea มีประโยชน์ในการรักษาผู้ป่วยภาวะ β-thalassemia/Hemoglobin E disease (β-thal/HbE) โดยเพิ่มระดับ HbF และความเข้มข้นเลือด Hemoglobin

คำสำคัญ : ● *β- thalassemia* ● *Hydroxyurea* ● *Hemoglobin F-stimulation*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2560;27:151-8.

Introduction

β-thalassemia refers to a heterogeneous group of blood disorders characterized by a decrease or lack of β-globin chain synthesis. β-thal/HbE is considered one of the major β-thalassemia syndromes in Southeast Asia.[1] The heterogeneity in the clinical manifestation of patients with β-thal/HbE may be explained by the nature of mutations in β-globin gene itself, the interaction with α-thalassemia gene and the difference in the amount of fetal Hb (Hb F) production, partially associated with Xmm I genotype homozygosity.[2]

Hydroxyurea (HU) is known to increase Hb F level. Related studies on HU therapy in adults with sickle cell anemia and homozygous β-thalassemia provided information concerning safety profiles, hematological effects and clinical benefits.[3,4] The first study reported using HU at a dosage of 20 mg/kg daily for 20 weeks to treat β-thal/HbE disease demonstrated a significant increase in Hb F levels, along with reciprocal decline in Hb E levels. The regimen showed modest effects on hemoglobin (Hb) and hematocrit (Hct) levels[5]. However, the effects of long term treatment with HU among adults with β-thal/HbE disease have not been reported. The purpose of this phase II study was to evaluate the effects of long term treatment with low-dose HU on Hb F level and other hematological parameters, including evaluation of quality of life (QOL) during the treatment period.

Materials and Methods

Patients

Twenty patients with β-thal/Hb E disease from Ramathibodi Hospital in Bangkok, Thailand entered this study. The hospital IRB approved the study, and the patients submitted written informed consent forms. Both splenectomized and nonsplenectomized patients with β-thal/HbE were eligible for the study. The study was restricted to patients who were not dependent on transfusions and who did not exhibit any severe liver or kidney dysfunction. Baseline Hb and hematocrit (Hct) levels, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and absolute reticulocyte counts were evaluated with a standard automatic blood cell analyzer (Technicon H3) before beginning the trial. A semiautomatic hemoglobin testing system was used to measure the relative and absolute levels of Hb F and Hb E using the HPLC technique (Bio-Rad, Variant system). Restriction endonuclease analysis of genomic leukocyte DNA was performed to assess the alpha-globin genotype and the haplotype of the beta-globin gene cluster. Factors affecting oxygen affinity of hemoglobin were evaluated by measuring levels of 2, 3-diphosphoglyceric acid (2, 3-DPG) and P_{50} at the beginning and at the end of the study. P_{50} indicates the PO_2 at which 50% saturation occurred.

HU treatment was started at 10 mg/kg/day once daily, for five consecutive days weekly. This dose of HU was chosen based on evaluation of the safety and tolerability determined in the phase I study.[5] During the treatment period of 36 months, patients were regularly monitored at 4-week intervals to assess toxicity, clinical adverse events, compliance and changes in Hb and Hct levels, MCV, MCH and absolute reticulocyte counts. A QOL assessment was conducted by questionnaires that reviewed physical-related, psychological-related, social-related and treatment-related domains. The questionnaires consisted of the biodermographic data tool, the QOL tool and the treatment satisfaction tool. The QOL tool was used to verify the consistency among normal subjects and thalassemic patients with variation in degree of anemia.

Statistical analysis

Statistical methods used in the study included the paired Student's *t* test for data with a Gaussian distribution and the Mann-Whitney U test for data with a non-Gaussian distribution. Repeated measures-analysis of variance (ANOVA) with Bonferroni's correction was also used to analyze multiple measurements within the same individual.[6]

To obtain the composite QOL score, the total QOL

score for each individual was computed from the mean scores of the four subscales. Reliability of the QOL tool was analyzed for test-retest stability (Pearson's correlations) and internal consistency (Cronbach's alpha coefficient). The *t*-statistic was used to analyze the QOL ratings.

Results

Clinical data

Twenty patients with β-thal/Hb E disease consecutively volunteered for the study. Among 20 subjects were 11 males and 9 females, ranging in age from 20 to 50 years (mean 30.1 ± 10.1 years). Fourteen of the 20 patients were splenectomized. Cases were recruited in this study consecutively. One patient was a viral hepatitis B carrier and two patients were seropositive for hepatitis C virus. All patients tested negative for HIV.

Laboratory data

Eight patients exhibited β^{17}/β^{E}mutation in the β-thalassemia gene, while the other eight exhibited the β^{4bp}/β^{E} mutation. The other patients exhibited the $\beta^{IVSII-654}/\beta^{E}$, β^{IVSI-5}/β^{E}, $\beta^{71/72}/\beta^{E}$ and the $\beta^{Capsite}/\beta^{E}$ mutations. Table 1 exhibits average Hb and Hct levels, relative percentage of Hb F and absolute amount of Hb F, MCV and ANC after 0, 12, 24, and 36 months of treatment. After 6 months, an averagely increase of 21.4% was observed in the relative percentage of Hb F and 11.8% in absolute Hb F levels. This response had persisted throughout the remainder of the 36-month study period. At the end of the study, the relative percentage of Hb F increased from a baseline value of 24.27 ± 11.91% (mean ± SD) to 28.80 ± 16.71%, accounting for a significant rise of 22.31% on average over the baseline levels ($p < 0.001$). The absolute amount of Hb F levels increased from 1.69 ± 1.20 g/dL to 1.89 ± 1.08 g/dL after one year and to 2.37 ± 0.97 g/dL after three years, accounting for 11.83% and 40.23% over the baseline levels, respectively ($p < 0.001$). The effects of HU treatment on relative and absolute Hb F levels are shown in Figure 1 and 2, subsequently. The average change in relative percentage of Hb F for all patients is shown in Figure 3. A reciprocal decrease was observed in the relative percentage of Hb E levels from 67.66 ± 16.11% to 62.67 ± 16.50% ($p < 0.001$). The MCV increased from 62.3 ± 8.24 fL to 68.31 ± 8.25 fL ($p < 0.05$). A modest increase in total Hb levels was also observed. No significant change in the number of white blood cells,

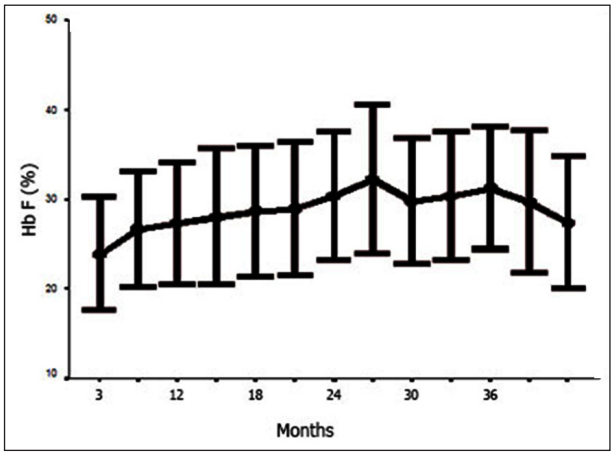

Figure 1 Effect of hydroxyurea (HU) treatment on the relative percentage of fetal hemoglobin (HbF) levels in patients with β-thalassemia/HbE. Subjects received HU treatment as described in the text. The relative percentage of Hb F was determined every 4 weeks. Data are expressed as average ± standard deviation.

Table 1 Hematologic values in patients with β-thalassemia/Hb E during treatment with hydroxyurea

	Baseline (n = 20)	12 months (n = 20)	24 months (n = 17)	36 months (n = 17)
Hb (g/dL) (mean ± SD)	6.15 ± 0.91	6.32 ± 1.03	6.27 ± 0.72	6.71 ± 0.93
% HbF (mean ± SD)	24.27 ± 11.91	29.51 ± 13.65	29.84 ± 13.61	28.80 ± 16.71
Absolute HbF (mean ± SD)	1.69 ± 1.20	1.89 ± 1.08	2.51 ± 1.12	2.37 ± 0.97
MCV (fL) (mean ± SD)	62.3 ± 8.24	66.55 ± 8.10	66.80 ± 7.63	68.31 ± 8.25
ANC (10^9/L) (mean ± SD)	4.20 ± 2.36	3.96 ± 2.09	3.32 ± 1.73	3.55 ± 1.82

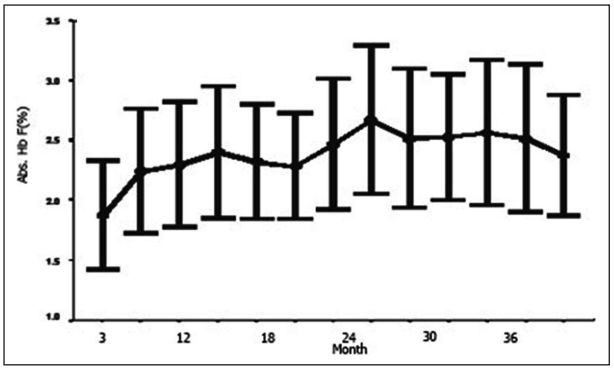

Figure 2 Effect of hydroxyurea (HU) treatment on absolute fetal hemoglobin (Hb F) levels in patients with β-thalassemia/HbE disease. Subjects received HU treatment as described in the text. The absolute levels of Hb F were determined every 4 weeks. Data are expressed as average ± standard deviation.

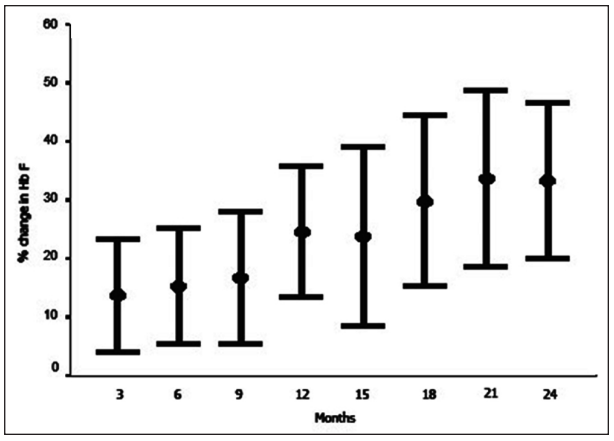

Figure 3 Mean increment in Hb F levels under HU treatment. The average percentage change in Hb F levels from baseline values is shown for the entire group of patients.

absolute number of neutrophils and reticulocytes was found. (Table 1)

The serum levels of ferritin, transferrin receptor (FT), and $^{G}\gamma/^{A}\gamma$ ratio before and after 12 and 36 months of HU treatment are exhibited in Table 2. Although none of the patients received iron chelators during the study period, the serum ferritin levels dropped from 3,778 mg/mL to 2,224 mg/mL and 2,047 ± 2,322 mg/mL after 12 and 36 months, respectively. Four patients required blood transfusions during a period of minor infections, mainly from respiratory tract infection. Improvement of erythrocytosis by HU treatment was demonstrated by a marked decline in serum Tf R levels, from 45.39 ± 19.3 mg/mL to 32.52 ± 17.18 mg/mL in the 36-month period. Moreover, this study revealed a tendency for the $^{G}\gamma/^{A}\gamma$ ratio to be elevated by HU treatment from the baseline level of 1.17 ± 0.17 to 1.29 ± 0.16 and 1.42 ± 0.22 at 12 and 36 months, respectively.

Hb F response and $^{G}\gamma/^{A}\gamma$ globin chain ratio stratified by XmnI polymorphism are demonstrated in table Table 3. Patients, with heterozygous for the XmnI polymorphism (-/+) had higher baseline HbF levels than those with homozygous Xmn (-/-). Patients responded to HU treatment with varying increases in absolute Hb F levels. Absolute Hb F levels increased by more than 50% from baseline levels in 11 patients, while the other 9 patients exhibited a more modest increase in absolute Hb F levels (< 50%). No correlation between baseline

Table 2 Effect of HU treatment on serum ferritin levels, serum transferrin receptor (Tf R) levels, $^{G}\gamma/^{A}\gamma$ ratio, 2, 3-diphosphoglyceric acid (DPG), whole blood DPG and P50

	Baseline	(mean ± SD)		
		12 months	36 months	p value
Serum ferritin (mg/mL)	3778 ± 2413	2,224 ± 1,357	2,047 ± 2,322	NS
Serum transferrin receptor	45.39 ± 19.37	34.44 ± 16.16	32.52 ± 17.18	$p < 0.05$
$^{G}\gamma/^{A}\gamma$	1.17 ± 0.7	1.29 ± 0.16	1.42 ± 0.22	$p < 0.05$
DPG (m mol/g)	22.76 ± 3.00	21.96 ± 2.31	22.25 ± 2.53	NS
Whole blood DPG	1.33 ± 0.18	1.35 ± 0.31	1.34 ± 0.26	NS
P50	25.21 ± 1.76	24.77 ± 2.16	24.41 ± 1.84	NS

Table 3 Hb F response and $^{G}\gamma/^{A}\gamma$ globin chain ratio stratified by XmnI polymorphism

	XmnI - +		XmnI - -	
	Hb F (%)	$^{G}\gamma/^{A}\gamma$	Hb F (%)	$^{G}\gamma/^{A}\gamma$
Baseline	30.22 ± 8.77	1.255 ± 0.08	13.86 ± 9.16	1.088 ± 0.14
12 month	34.82 ± 9.19	1.30 ± 0.17	18.58 ± 13.14	1.252 ± 0.12
24 month	37.15 ± 8.17	1.386 ± 0.2	19.04 ± 12.5	1.27 ± 0.12
36 month	33.63 ± 9.63	1.442 ± 0.14	15.2 ± 13.5	1.254 ± 0.16

Table 4 Predictors for Hb F response to HU therapy: Hb F response was stratified by Hb F increment from the baseline to 51-100% increment, 26-50% increment and 0-25% increment.

Hb F increment	N	Baseline HbF (%)	Baseline Absolute HbF(g/dL)	XmnI -/+ : -/-	HbF(g/dL) increment	$^{G}\gamma/^{A}\gamma$ increment
0-25	9	27.65 ± 4.54	1.92 ± 0.32	7:2	12.93 ± 2.54	0.55 ± 0.34
26-50	6	20.81 ± 6.07	2.22 ± 0.42	3:3	31.11 ± 2.31	0.52 ± 0.27
51-100	5	26.44 ± 1.47	1.34 ± 0.09	4:1	75.84 ± 7.9	0.26 ± 0.16

Table 5 Reported quality of life in patients with β thalassemia/Hb E disease before and after 36 months of treatment

Domain	Before (Mean ± SD)	After (Mean ± SD)	p-value
Physical	24.05 ± 4.02	25.73 ± 3.63	0.022
Psychological	25.05 ± 7.29	27.42 ± 5.88	< .001
Social	26.89 ± 5.93	27.10 ± 5.54	< .001
Treatment-related	26.31 ± 4.66	28.42 ± 3.51	< .001

Hb F levels, splenectomy status and XmnI polymorphism was observed (Table 4). Three patients with low baseline levels of relative Hb F percentage (less than 6%) also responded to HU therapy.

Clinical response to HU

Most patients experienced improvement in their sense of well-being, increased exercise tolerance with significant increase in QOL in physical, mental, social and treatment-related domains ($p < 0.05$), as shown in Table 5. Three patients, receiving periodic blood transfusions responded to HU therapy and no longer required transfusions.

Side effects and complications

All patients tolerated the HU treatment very well and exhibited good compliance. No adverse effects were observed and no patients exhibited febrile neutropenia or thrombocytopenia. ANC was slightly decreased without significance. No severe infection complication was noted in any patients. Treatment was stopped in three patients after 18 months. Of these, two post-splenectomized patients developed pulmonary hypertension, which is a known complication in post-splenectomized thalassemic patients.[7, 8] The third patient developed acute lymphoblastic leukemia without any evidence of secondary leukemia.

Discussion

The pharmacological induction of Hb F is an alternative approach to improve the clinical condition of patients with homozygous β-thalassemia and β-thal/Hb E disease.[3-5,9-12] The induction of Hb F synthesis has been reported to have significant clinical benefits, including elimination of the need for transfusions in β-thalassemia patients.[9-14] HU treatment has previously

been reported to increase levels of Hb F, γ-globin and b-globin in some thalassemia patients.[15] Unfortunately, HU treatment alone was found to be ineffective in treating those patients with transfusion-dependent β-thalassemia.[12] However, a combination of HU and sodium phenylbutyrate therapy was introduced and found to be promising in treating patients with thalassemia intermedia, who failed to response after HU alone therapy.[16] In a phase I study of patients with β-thal/Hb E disease, treatment with HU at doses ranging from 10 to 20 mg/kg/day resulted in a 32% rise in Hb F levels.[5] Our phase II study also demonstrated that long term low-dose HU therapy could be beneficial to those with β-thal/Hb E disease. This regimen significantly increased relative and absolute Hb F levels, with a reciprocal decline in Hb E level after 6 months of HU therapy. Hb F level reached to its peak level after 6-12 months after starting HU treatment. The responses had been persistently achieved throughout the study period. From our study, long term low-dose HU treatment were shown to significantly augment Hb F production and to increase absolute total Hb F concentrations by as much as 26% from baseline. None of the clinical or genetic parameters that were evaluated, including XmnI polymorphism status, could predict Hb F response. Although, a slight elevation in total Hb concentrations was noted, a parallel reduction in serum TfR levels was consistent with even more effective erythropoiesis.[8] Despite the modest increase in Hb levels and unchanged Hb affinity, improvement in QOL was demonstrated after treatment with HU. These findings could be explained by the reduction of ineffective hematopoiesis. Even though the maximal tolerated doses were still unknown, increasing the dose of HU to 20 mg/day might constitute a practical tactic to succeed in treating nonresponders. The result of this prospective cohort study was limited due to the small number of enrolled patients and study characteristics. This study illustrated the safe use of low dose hydroxyurea in thalassemic patients. Further studies especially those conducted with randomized control trials in a heterogenous population should be encouraged to link the result to clinical use.

The possible risk of malignancy and leukemogenesis is a major concern in long term therapy with HU. Long term HU therapy was reported to be effective for treating sickle cell anemia and β-thalassemia without increasing the risk of leukemia.[7] The incidence of acute leukemia in patients who were treated with HU for myeloproliferative disorders was comparable to that observed in the normal population.[17] However, the patient who developed acute lymphoblastic leukemia in this study did not exhibit any evidence of secondary leukemia. In conclusion, long term HU treatment in adults with β-thalassemia/Hb E appears to be safe and practical.

Summary

Long term use of hydroxyurea for treating patients with β-thalassemia/Hb E disease was able to provide increased absolute Hb F levels, decreasing ineffective erythropoiesis. With these effects, these patients were able to achieve a better QOL and improved health without any serious side effects. This study validates the use of hydroxyurea as a safe and effective therapy for β-thalassemia/Hb E disease.

Acknowledgements

This study was supported by research grants from Mahidol University and from The Thai Research Fund (TRF)

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