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Long-term hydroxyurea therapy in beta-thalassaemia patients

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Abstract: *Objective:* The study aimed to investigate the use of hydroxyurea (HU) for the treatment of beta-thalassaemia (β-thal) patients. *Methods:* We examined the haematological effects of orally administered HU (10–20 mg/kg/d) in 11 patients, including four β-thal major and seven β-thal intermedia patients. Complete blood count and levels of foetal haemoglobin (HbF), liver enzymes and serum creatinine were evaluated before and during HU. Response to therapy was evaluated at 6 months of treatment. *Results:* A substantial increase in haemoglobin (Hb) level (4.1 g/dL), leading to complete withdrawal from a regular transfusion programme, was observed in one unique β-thal major patient. In the β-thal intermedia patients, increases in Hb level of 1.3, 1.9 and 2.0 g/dL were observed in three of seven (42.9%) patients during HU therapy. The mean values of Hb, mean corpuscular haemoglobin (MCH), and HbF were higher during HU treatment than baseline values (8.7 vs. 7.7 g/dL, $P = 0.05$; 26.7 vs. 22.9 pg, $P = 0.05$; 57.2 vs. 44.9%, $P = 0.04$; respectively). In contrast, the mean reticulocyte count measured during therapy decreased (97.0×10^9 vs. 632.0×10^9/L, $P = 0.03$). No correlations were observed between levels of Hb and HbF ($r = 0.77$, $P = 0.10$), and levels of Hb and reticulocyte counts ($r = 0.26$, $P = 0.31$). No significant toxicity was observed in our patients. *Conclusion:* These results suggest that HU may improve Hb levels in β-thal. Thus, we may conclude that a large trial concerning the response to HU in these patients should be carried out to clarify this issue.

Erich Vinicius de Paula[1], Carmen Silvia Passos Lima[1], Valder Roberval Arruda[2], Fernando Lopes Alberto[1], Sara Teresinha Ollala Saad[2], Fernando Ferreira Costa[2]

[1]Haematology and Haemotherapy Centre, and [2]Department of Internal Medicine, State University of Campinas, Campinas, São Paulo, Brazil

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Correspondence: Fernando Ferreira Costa MD, PhD, Hemocentro-Unicamp, Cidade Universitária `Zeferino Vaz', Barão Geraldo, Campinas (CP: 6198, Cep.: 13081-970), São Paulo, Brazil
Tel: +55 19 3788 4739
Fax: +55 19 3289 1089
e-mail: ferreira@unicamp.br

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Beta-thalassaemia (β-thal), the most commonly inherited blood disorder in the world, results from a number of genetic defects in β-globin gene expression (1). Deficient β-globin production determines an imbalance in the α/β-chain ratio and excess α-chains precipitate within the red blood cells (RBC) resulting in haemolysis. The phenotypic presentation varies, but two forms of severe β-thal can be identified, major and intermedia, which generally correlate with the degree of α/β imbalance (1).

Patients with β-thal major require regular RBC transfusion to sustain their lives, whilst the β-thal intermedia patients have sufficient RBC production to sustain their haemoglobin (Hb) levels between 6 and 9 g/dL. Often, regulars transfusion therapy is delayed in such individuals, thereby minimising iron overload at the expense of anaemia, with consequent cardiomegaly and bone deformities. An increase, even modest, in RBC production with suppression of bone marrow-turnover might have a beneficial clinical effect on these patients.

It has been suggested that increased levels of foetal Hb (HbF) could improve the clinical features of β-thal patients, because of an improvement in the balance of globin synthesis (2–4). Hydroxyurea (HU) is a well known agent capable of stimulating HbF production in sickle cell disease (5–8) and β-thal patients (9) by a not completely understood mechanism. It may select and recruit the early red cell progenitors, which maintain the programme for γ-chain synthesis (8, 9), and also may stimulate the gamma-globin gene expression on the late red cell progenitors (8, 9). However, the overall results presented on anecdotal reports and small series of

patients treated with HU (10–25) were ambiguous with respect to its clinical effects in β-thal.

As the usefulness of HU in the treatment of the β-thal patients has still not been established, this was the aim of the study presented herein.

Material and methods

Eligibility requirements

Patients with β-thal major or β-thal intermedia in attendance at the Haematology and Haemotherapy Centre of the University of Campinas, who presented no medical problems apart from those associated with their conditions, were considered as fully reliable for HU treatment. The diagnosis of the disease was based on clinical, familial and laboratory data, including electrophoresis on cellulose acetate at pH 8.9 and on agar gel at pH 6.2, and the estimation of HbF and HbA2 (26). The mutations of the β-globin gene cluster were detected with the dideoxy chain termination sequencing method (27). The study protocol was in accordance with the Helsinski Declaration of 1975 and was approved by the local Ethics Committee.

Study protocol

The initial dose of HU (Bristol, Regensburg, Germany) was 10 mg/kg/d, given once a day, and was increased in increments of 5 mg/kg/d every 8 wk to a maximum of 20 mg/kg/d, or until toxicity developed. Haematological toxicity was defined by an absolute reticulocyte count $<50.0 \times 10^9$/L, an absolute neutrophil count $<2.0 \times 10^9$/L, or platelet count $<100.0 \times 10^9$/L. Hepatic and renal toxicity was defined by a twofold increase in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) or s >50% increase in serum creatinine level. If toxicity occurred, treatment was stopped until blood counts returned to normal and then reintroduced at a lower dose which, if tolerated, was considered the maximum tolerated dose. HU was discontinued in non-responding patients after treatment with the dose of 20 mg/kg/d. All patients received folate supplementation for at least 6 months prior to initiating HU and during HU therapy.

Laboratory monitoring

Before starting HU, all patients underwent laboratory testing including a complete blood count, HbF estimation and serum ALT, AST and creatinine levels, which were considered as the baseline values. Complete blood count was carried out using an automated instrument (Cell-Dyn, Model 1700, Abbott, Abbott Park, IL, USA). Reticulocyte count and the estimation of HbF were performed using the microscopic brilliant cresyl blue method (28) and alkali denaturation method (29), respectively. Baseline Hb values were calculated as an average of three values immediately preceding the initiation of HU.

During treatment, a complete blood count was performed every 2 wk and a chemistry panel was obtained every 4 wk until the maximum HU dosage was established. Thereafter, all measurements were checked monthly.

A sustained increase of at least 1.0 g/dL in Hb level in non-transfusion-dependent patients was considered as a response to therapy (30), or a 50% or more decrease in transfusion needs, measured after 6 months. Thereafter, the same evaluation was performed every 6 months up to the time of HU interruption, with the purpose of verifying whether an additional response occurred during longer HU treatment.

Statistical analysis

The Wilcoxon test was used to compare the differences between initial and final measurements. Correlation between levels of Hb, HbF, Mean Corpuscular Volume (MCV), MCH, and reticulocyte numbers was tested using the Spearman's coefficient (31).

Results

Eleven patients with β-thal major and intermedia were included in the study. The median follow-up during HU therapy was 18 months (range: 6–96 months) (Table 1). Only one patient was submitted to splenectomy after HU treatment (case 3).

Patients with β-thal major

A substantial increase in Hb level (3.0 g/dL) above the baseline value, despite continuation of the same regular RBC transfusion schedule, was observed in one patient (case 1) after 3 months of 10 mg/kg/d of HU. Nine months later, transfusion was stopped altogether, with no further major variations in Hb levels. The clinical and haematological features of this case have been presented previously by our group (15). In the most recent follow-up, the patient was without a transfusion for 8 yr whilst on HU therapy. During this period his Hb level remained between 10.6 and 11.9 g/dL. In contrast, the administration of the HU to the remaining three patients failed to show any increase in Hb levels or decrease in the RBC transfusion requirements. Neutropenia (neutrophil count $= 1.3 \times 10^9$/L) was

Table 1. Clinical characteristics of 11 beta-thalassaemia major and intermedia patients included in the study and the duration of the hydroxyurea treatment

Case	Age (years)	Sex	Genotype	Phenotype	HU treatment (months)
1	17	Male	β^0 39/β^0 39	Major	96
2	22	Male	β IVS-I-110/β IVS-I-6	Major	12
3	19	Female	β^0 39/β^0 39	Major	6
4	16	Male	β^0 39/β^0 39	Major	24
5	27	Female	β IVS-I-110/δβSicilian	Intermedia	12
6	35	Male	β IVS-I-110/β IVS-I-6	Intermedia	44
7	35	Male	β IVS-I-110/δβSicilian	Intermedia	69
8	38	Male	β IVS-I-6/-87	Intermedia	24
9	16	Female	β IVS-I-1/β IVS-I-6	Intermedia	18
10	68	Male	–101/β^0 39	Intermedia	12
11	35	Female	β^0 39/NI	Intermedia	18

HU, hydroxyurea; NI, not identified.

the only side-effect observed in one patient (case 3) when 20 mg/kg/d HU was administered, and was rapidly reversed upon discontinuation of the treatment.

Patients with β-thal intermedia

The baseline haematological values and those obtained after 6 months of HU treatment are presented in Table 2.

Increases in Hb level of 1.3, 2.0 and 1.9 g/dL were observed in three of seven (42.9%) β-thal intermedia patients (cases 6, 7 and 8) during HU treatment. Responding doses were 10 mg/kg/d for the first patient and 15 mg/kg/d for the others. Further upward dose adjustments did not enhance responses in any of them. Additional increase in Hb level (1.2 g/dL) was obtained in one responding patient (case 6) at 12 months of treatment. Therapy longer than 12 months did not cause beneficial effects for any of the studied patients (Fig. 1). All β-thal intermedia patients had received only sporadic RBC transfusion before HU and only one unique non-responding patient (case 10) required two units of RBC during therapy. Decrease in reticulocyte number was observed in all six analysable patients. MCV increased in five of seven patients, including all responders and two non-responding patients (cases 5 and 11). MCH did not increase in one unique non-responding patient (case 10). Increases in HbF levels were found in four of six analysable patients, including two responders (cases 6 and 8) and two non-responding (cases 9 and 11) patients.

The mean values of Hb, MCH and HbF measured after 6 months of HU therapy were higher than baseline values (8.7 ± 1.8 vs. 7.7 ± 1.3 g/dl, $P = 0.05$; 26.7 ± 1.9 vs. 22.9 ± 1.8 pg, $P = 0.05$; 57.2 ± 34.8 vs. 44.9 ± 41.4%, $P = 0.04$; respectively). Similar mean values of MCV were found in analyses performed after and before treatment (76.9 ± 4.7 vs. 70.3 ± 5.8 fl, $P = 0.10$). In contrast, mean reticulocyte count obtained after treatment was lower than baseline values ($97.0 \pm 53.0 \times 10^9$ vs. $632.0 \pm 605.0 \times 10^9$/dL, $P = 0.03$).

No correlation was observed between levels of Hb and MCV values ($r = 0.14$, $P = 0.38$), MCH values ($r = 0.64$, $P = 0.06$), reticulocyte count ($r = 0.26$, $P = 0.31$) and HbF levels ($r = 0.77$, $P = 0.10$). Values of MCV and HbF levels were also not correlated ($r = -0.08$; $P = 0.79$).

Reticulopenia (reticulocyte count = 44.0×10^9/dL and neutropenia (neutrophil count = 1.2×10^9/L) were observed in one patient (case 4) and in one other patient (case 6), respectively, during HU at a dose of 20 mg/kg/d. The manifestations were rapidly reversible upon discontinuation of HU and did not recur upon restart. No hepatic or renal toxicity was observed in the patients studied.

Table 2. Haematological changes in seven beta-thalassaemia intermedia patients obtained before and after 6 months of hydroxyurea therapy

	Hb (g/dL)		Ret ($\times 10^9$/L)		MCV (fl)		MCH (pg)		HbF (%)	
Case	Baseline	HU	Baseline	HU	Baseline	HU	Baseline	HU	Baseline	HU
5	9.9	10.8	1680.0	220.0	71	74	23.2	27.3	97.2	98.0
6	8.5	9.8	294.0	72.0	68	77	21.4	28.6	20.9	48.0
7	8.4	10.4	1000.0	90.0	78	83	24.9	28.8	98.4	98.4
8	7.2	9.1	519.0	44.0	66	79	20.5	26.7	23.3	30.0
9	6.4	6.8	90.0	80.0	73	72	23.9	24.8	23.0	55.0
10	6.5	7.1	NA	NA	75	71	24.9	23.7	NA	NA
11	7.0	6.5	210.0	97.0	61	82	21.3	27	6.7	14.0

Hb, haemoglobin; Ret, reticulocyte; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; HbF, foetal haemoglobin; HU, hydroxyurea; NA, not avaliable.

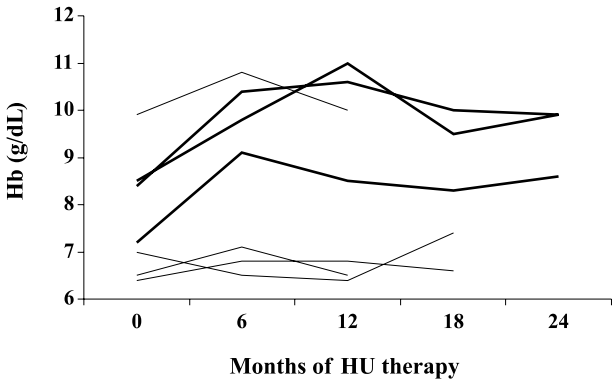

Fig. 1. Changes in total haemoglobin obtained from seven beta-thalassaemia intermedia patients before and every 6 months during hydroxyurea treatment. Thick lines indicate responding patients.

Discussion

Although HU successfully increases Hb and HbF in patients with sickle cell disease (6–8), there is limited experience with the agent in β-thal. We reported the results of the treatment of four patients with β-thal major and seven patients with β-thal intermedia with HU. The effects on total Hb and transfusions requirements were the main measure of treatment efficacy.

We found that the clinical response was highly variable, in accordance with previous reports (9–25).

In the β-thal major patients, a substantial and persistent increase in Hb level almost up to normal values, without transfusion, was observed in one case. However, treatment failed to induce any increase in Hb levels or decrease in the RBC transfusion requirements in the remaining patients. These results and those obtained from other studies with a small number of patients (10, 11, 16, 17) suggest that responses to HU therapy may be expected in this form of the disease.

In the β-thal intermedia patients, we found a mean increment in Hb level of 1.7 g/dL measured at 6 months of therapy in three of seven patients, who received HU at doses of 10–15 mg/kg/d. No other responding patients were observed when the dose was increased to the maximum allowed dose. Only one previous responding patient presented an additional increase in Hb level measured at 12 months of therapy. These results and others obtained from studies with a small number of patients (11–15, 18–25) suggest that patients with this form of the disease can obtain beneficial effects from HU therapy after a relatively short period of time.

We did not find a correlation between increase in Hb level and decrease in reticulocyte number. Therefore, the increase in Hb levels found in our cases seemed not to be determined only by the decreased haemolysis. As ineffective erythropoiesis underlies the thalassaemic defect (1), and HU may improve the balance of globin synthesis (13), it is possible that the increase in Hb levels in our cases had been determined by both mechanisms.

On the other hand, increases in HbF values did not always correlate with an increase in clinical response. Although four analysed patients presented a substantial increase in HbF percentages, the concomitant rise in Hb level was not seen in two of them. Furthermore, increase in HbF levels was not seen in one responding patient. Other reports have conflicted in this regard. A few studies including a small number of patients have reported a positive correlation between Hb and HbF (11, 12, 14, 15, 19–21, 24, 25) while others have shown that some patients with increased HbF have no response in Hb after treatment with HU (11, 18, 21, 22). In addition, increase in Hb levels without increase in levels of HbF were also found in other studies (13, 17). This suggest that HU may act in a non-selective manner and affect more than γ-globin production alone. In fact, one study demonstrated that HU can determine increases in α-, β- and γ-globin levels (13), and hence it may promotes increase in HbA levels and reduction in the ineffective erythropoieisis of thalassaemic deffect. Furthermore, the relative increases in individual globins may be influenced by the particular β-globin genetic defect and other genetic modifiers.

Assuming that HU may determine a clinical benefit in β-thal patients raises the questions about its safety. With the exception of reversible myelosuppression with doses of greater than 15 mg/kg/d, HU had no short-term toxicity in our study and in others previously conducted (12, 18, 19, 21). However, its long-term safety with regard to leucaemogenesis remains to be clarified. The current experience of HU in sickle cell disease patients has strengthened its safety, although continuous follow-up is necessary to draw unequivocal conclusions (32).

The HU was easy to use and well-tolerated in our patients. In addition, responses to therapy occurred in the first 6 months of starting HU. Therefore, we may conclude that a large trial concerning the response of HU in β-thal should be carried out to clarify this issue.

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