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Alpha thalassaemia and response to hydroxyurea in sickle cell anaemia

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Abstract

Background—Hydroxyurea (HU) reduces vaso-occlusive crises (VOC) and other complications of sickle cell anaemia (SCA). Alpha thalassaemia is a known modifier of SCA. Studies on the efficacy of HU in SCA patients with α -thalassaemia have yielded varying results.

Objective—To determine the effect of α -thalassaemia on response to HU therapy in the Multicenter Study of Hydroxyurea (MSH) cohort.

Methods—We compared the laboratory parameters and VOC incidence in the MSH cohort stratified by the presence or absence of α -thalassaemia.

Results—HU showed significant ($p = 0.001$ for all baseline vs. follow-up comparisons) treatment effect on red cell indices irrespective of α -globin gene deletion. The magnitude of the HU-related changes was similar for MCV (no α -thalassaemia 13 fl, α -thalassaemia 13 fl), and MCH (no α -thalassaemia 4 pg, α -thalassaemia 4 pg) in both groups. Foetal haemoglobin (HbF) and F cells also increased significantly with HU treatment in both groups. Total hemoglobin increased after HU treatment in both groups but the increase was smaller and not statistically significant in α -thalassaemia patients. In contrast, HU-related reduction of VOCs was more pronounced in patients with α -thalassaemia (VOC incidence rate ratio HU/placebo: 0.63 for α -thalassaemia vs. 0.54 for no α -thalassaemia (p for interaction 0.003).

Conclusion—HU decreases VOCs in SCA patients with and without α -thalassaemia and the degree of VOC reduction was more pronounced in the patients with alpha thalassaemia. Despite

lower baseline values, changes in standard laboratory parameters such as MCV, and HbF percent remain useful in monitoring HU therapy in presence of α -thalassaemia.

Keywords

Sickle cell anaemia; Hydroxyurea; Alpha thalassaemia; Vaso occlusive pain crisis

Introduction

Sickle cell anaemia (SCA) is characterised by the presence of haemoglobin S (HbS). Polymerisation of HbS occurs during deoxygenation and causes erythrocyte rigidity and sickling, one of the main underlying pathophysiologic mechanisms leading to microcirculatory obstruction and painful vaso-occlusive crisis (VOC).

Foetal haemoglobin (HbF) inhibits the polymerisation of deoxy-haemoglobin S and thus exerts a beneficial effect in SCA. Hydroxyurea (Hydroxycarbamide, HU) administration increases foetal haemoglobin in SCA (1). Two randomised double-blind placebo-controlled trials, the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH), and the BABY HUG study, showed that hydroxyurea reduced the number of VOCs, acute chest syndrome, and the need for red cell transfusions in SCA patients (1–3). Laboratory markers such as an increase in foetal haemoglobin and erythrocyte mean corpuscular volume (MCV), and a decrease in neutrophil and reticulocyte counts were associated with hydroxyurea administration and are helpful in assessing therapy adherence (2). Response to hydroxyurea therapy varies among individuals with SCA (4).

Alpha thalassaemia is characterised by deletion of at least one α -globin gene and about one-third of sickle cell patients of African descent co-inherit at least one α -gene deletion (5). The presence of alpha thalassaemia impacts red cell pathobiology in sickle cell disease by lowering mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) (6), which decreases intra-erythrocytic concentration of HbS. This, in turn leads to less haemoglobin polymerisation, and to better cell hydration and deformability. Also, the greater membrane surface area/cell volume ratio in the erythrocytes of SCA- α -thalassaemia patients diminishes membrane stretching and cation loss, which, along with decreased Hb S polymerization, improve red blood cell (RBC) life span (5, 7). Despite the beneficial effect of α -thalassaemia on erythrocytes, its clinical effects are mixed: while the longer survival of sickle RBC improves the anaemia, the higher hematocrit is associated with more frequent VOCs, presumably due to an increased blood viscosity (8–10).

Whether and to what degree the coinheritance of α -thalassaemia modifies the effect of HU therapy in SCD is a clinically important question, and previous studies of the issue have yielded diverging results. In a prospective open label study, Vasavda et al reported significantly attenuated response to hydroxyurea treatment in SCA/ α -thalassaemia patients. These authors showed a reduction in hospitalisation days for pain in SCA individuals, both with and without α -thalassaemia. However, this effect was significantly smaller in SCA/ α -thalassaemia than that in SCA subjects without α -thalassaemia (11). The SCA/ α -thalassaemia group also had higher levels of plasma cell free DNA, a marker of tissue damage, levels that decrease with hydroxyurea therapy (12). Laboratory responses associated with hydroxyurea therapy including total haemoglobin, HbF, MCV, and MCH, were also attenuated in SCA/ α -thalassaemia, leading the authors to conclude that α -thalassaemia blunts the laboratory and clinical response to hydroxyurea (11, 13). It was also speculated that some of the hydroxyurea non-responders reported in the MSH trial (4) could have been carriers of α -thalassaemia (6). In contrast, a recent analysis of the effect of

genetic modifiers of SCA on HU treatment response (BABY HUG data) found significant increases in haemoglobin, MCV, MCH, and % HbF, and decreases in reticulocyte count and bilirubin in SCA infants regardless of α -thalassaemia status (14). Furthermore, the BABY HUG data showed that the magnitude of the reduction in pain crises after HU treatment was greater in SCA infants with co-existing α -thalassaemia.

In the present report, we analysed data from the Multicenter Study of Hydroxyurea (MSH) to assess the role of α -thalassaemia on the clinical and hematologic response to hydroxyurea in adults with SCA.

Methods

MSH data was acquired from the NHLBI database repository after approval was obtained from the IRB at the Children's National Medical Center, Washington DC. The MSH study was designed to determine the efficacy of hydroxyurea in SCA. This double blind, placebo controlled, multicenter study recruited 299 patients with SCA (homozygous SS and sickle β^0 thalassaemia). Patients with coexistent α -thalassaemia were not excluded. Alpha globin genotype was ascertained by Southern blot analysis as previously described (4, 15, 16). Eligibility was limited to severely affected adults as defined by ≥ 3 VOCs per year because of uncertainty concerning acute and chronic toxicity related to hydroxyurea. Patients were randomised to hydroxyurea or placebo and were followed by clinic visits every two weeks. The drug dose was increased up to the maximum tolerated dose, or up to 35 mg/kg/day. Laboratory studies were obtained every two weeks to monitor myelotoxicity, a side effect of HU. The primary endpoint of the MSH was 50% or greater reduction in the rate of pain crisis in the hydroxyurea arm compared to the placebo arm, as a clinically significant indication of the effectiveness of HU.

Statistical considerations

We analysed the MSH data to determine the effect of hydroxyurea treatment on haemoglobin, MCV, WBC, reticulocyte count, and pain crisis rate in two different strata including patients without α -gene deletion (wild type) and those with one or two α -globin gene deletion (α -thalassaemia trait). In each group, Panel Data Analysis (repeated measure), using population average (Generalised Estimating Equation) models, was applied to compute the effect of HU. These models present the overall effect of hydroxyurea during the whole follow-up period. Then, these treatment effects were compared between two groups (based on α -gene status) using interaction term between α -gene status and hydroxyurea. All analyses were performed in Stata 12.0 (StataCorp, College Station, TX). P values < 0.05 were considered statistically significant.

Results

Baseline characteristics

A total of 299 patients were recruited in the MSH. As previously described baseline demographic and laboratory variables were not different in the hydroxyurea and placebo groups (1). In this cohort, 84 patients (28%) had single α -gene deletion and 7 (2%) had two α -gene deletions. These frequencies were evenly distributed between the hydroxyurea and placebo groups. At study entry, patients with α -gene deletion had higher haemoglobin and lower MCH, MCHC, MCV, and absolute reticulocyte counts, compared to patients without α -gene deletion. Due to MSH enrollment eligibility criteria all patients had ≥ 3 VOCs in the year before the enrollment. However, patients with SCA/ α -thalassaemia were overrepresented in the category of 6 to 9 VOCs per year (Table 1).

Stratified analysis of treatment effect

We evaluated the effect of hydroxyurea in the study population stratified by no α -gene deletion and one or two α -gene deletion (α thalassaemia trait) (Table 2). During the 104 weeks of follow-up, hydroxyurea significantly increased MCV, MCH and MCHC in both groups, while HU only increased haemoglobin level significantly in the group without α -gene deletion. Hydroxyurea also decreased WBC and ARC in both groups and platelets in the wild α -gene group. The effect of hydroxyurea in reducing ARC was less pronounced in the α -thalassaemia group. Data on HbF percent and F cell was available in the part of patients at baseline and during the follow-up as listed in Table 1 and 2. HU increased HbF and F cells in all patients. However, the increase was greater in patients without alpha thalassaemia (Table 2). Hydroxyurea significantly decreased the number of VOCs in both groups regardless of α gene deletion status. Test for interaction indicated that the effect of treatment in reducing the VOCs was more pronounced in patients with α -globin gene deletion (Table 2).

Discussion

While α -thalassaemia is a known modifier of SCA, studies have reported variable results on hydroxyurea response in SCA patients co-inheriting α -thalassaemia. We present data from the MSH cohort showing that hydroxyurea is effective in increasing MCV, MCH, MCHC, F cells and HbF % and decreasing ARC and WBC, as well as VOCs in the patients with and without α -thalassaemia. Interestingly, the reduction in VOCs was more pronounced in patients with α -globin gene deletion while the increase in haemoglobin was statistically significant only in SCA patients without α -thalassaemia. As expected, SCA/alpha thalassaemia patients at baseline had higher haemoglobin, lower reticulocytes and MCV. The MSH study was designed to include patients with relatively severe disease. Thus per enrollment criteria, all study participants had three or more VOCs in the preceding year. However, patients with SCA/ α -thalassaemia were overrepresented in the category of 6–9 VOCs per year confirming that these patients are at higher risk for frequent VOCs.

Similar to our results, trends for greater reduction of VOC in presence of alpha thalassaemia were observed also in the BABY-HUG cohort (14). We do not have an explanation for why the HU beneficial effect appears to be stronger in adults and children with SCA and α -thalassaemia. We speculate that this difference is related to their higher baseline VOC rate. In terms of laboratory findings, SCA/ α -thalassaemia patients had a lower MCV while on HU, which is likely due to their lower baseline MCV, since the degree of increase in MCV was not different between the groups. Similar changes in MCV were also observed in the BABY-HUG trial, where the degree of increase in MCV may be even higher in SCA/ α -thalassaemia group (14). The lower magnitude of the HU-related increases in haemoglobin and foetal haemoglobin in the presence of alpha thalassaemia are also unexplained. Possibly, the relative paucity of alpha globin chains could have disproportionately affected foetal haemoglobin synthesis. In any case, the difference in increase of HbF was not statistically significant between the groups. The more pronounced reduction in reticulocyte count in the non-thalassaemia group could be related to higher degree of hemolysis and reticulocytosis at baseline. In the MSH cohort, haemoglobin level at study entry was higher in presence of α -thalassaemia, as expected. However, this baseline finding was not observed in the BABY HUG cohort. We speculate that this discrepancy could be due to the age difference between the cohorts and/or may also reflect the effect of the spleen on shortening red cell survival in all SCA infants (17, 18) regardless of thalassemia status. We conclude that hydroxyurea is effective in reducing VOC pain crises in SCA individuals with and without α -globin gene deletion, confirming the current approach of prescribing hydroxyurea to patients with SCA regardless of their alpha thalassaemia status. Furthermore, given the greater reduction in VOCs, the clinical benefit may be even greater for SCA patients with alpha thalassaemia,

who typically experience a higher rate of VOCs. We also show that despite the differences at baseline, standard laboratory parameters can be utilised to monitor hydroxyurea therapy in SCA patients with alpha-thalassaemia.

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Table 1

Baseline variables by α -globin gene status. Results are in median (interquartile range) unless otherwise indicated.

	No α -globin gene deletion (n= 208)	α -globin gene deletion (n=91)	P value
Age (years)	30 (24–36)	29 (23–33)	0.07
Male gender	107 (51%)	36 (43%)	0.14
Weight (kg)	61 (54–69)	60 (56–71)	0.6
Haemoglobin (g/dL)	8.4 (7.4–9.2)	8.5 (8.0–9.4)	0.021
MCV (fL)	96 (91–101)	88 (82–93)	<0.001
MCH (pg)	33 (31–35)	30 (27–31)	<0.001
MCHC (g/dL)	33.2 (32.4–33.9)	32.9 (31.9–33.7)	0.011
WBC (k/mL)	12.3 (10.5–14.7)	12.1 (9.6–13.9)	0.13
Platelet count (k/mL)	452 (371–529)	458 (374–531)	0.9
Absolute reticulocyte count (k/mL)	332 (283–400)	277 (245–327)	<0.001
HbF (%)	4.5 (2.6–7.1)	4.2 (2.3–6.7)	0.3
F-cells (%)	32 (21–44)	31 (19–42)	0.3
Number of VOCs [†]			P = 0.021
3–5	98 (47%)	32 (35%)	
6–9	24 (12%)	21 (23%)	
10	86 (41%)	38 (42%)	

[†]In the year before enrollment. The frequency of 6–9 VOCs was significantly higher in α -thalassemia group (P=0.010, one degree of freedom)

Table 2

Hydroxyurea treatment effect by α -globin gene status. Results are in mean (95% CI) change during the 104 weeks of treatment.

	No α -globin gene deletion (n=208)		α -globin gene deletion (n=91)		P for interaction**
	Treatment effect*	P value	Treatment effect*	P value	
Haemoglobin (g/dL)	0.81 (0.44–1.17)	<0.001	0.39 (–0.08–0.86)	0.11	0.21
MCV (fL)	13 (10–16)	<0.001	13 (9–17)	<0.001	0.9
MCH (pg)	4 (3–5)	<0.001	4 (3–6)	<0.001	0.5
MCHC (g/dL)	0.29 (0.05–0.53)	0.019	0.44 (0.04–0.84)	0.029	0.5
WBC (k/mL)	–3.0 (–3.8–2.2)	<0.001	–2.2 (–3.3–1.2)	<0.001	0.18
Hb-F (%)	4.20 (2.45–5.94) ¹	<0.001	2.96 (0.40–5.52) ²	0.023	0.4
F-cells (%)	16.20 (10.90–21.49) ³	<0.001	11.5 (3.52–19.45) ⁴	0.005	0.3
Platelet count (k/mL)	–46 (–80–12)	0.003	–26 (–73–20)	0.23	0.5
Absolute reticulocyte count (k/mL)	–121 (–145–98)	<0.001	–72 (–100–44)	<0.001	0.046
VOC incidence rate ratio HU/placebo	0.63 (0.47–0.84)	0.002	0.54 (0.39–0.76)	<0.001	0.003

* Treatment effect reflects the effect of hydroxyurea (HU) as measured by comparing parameters between the HU and placebo arms during the 104 weeks of treatment.

** P for interaction compares effect of HU in patients without and with α -globin gene deletion

- ¹ n= 156 with average 1.2 observation per patient
- ² n= 68 with average 1.1 observation per patient
- ³ n= 207 with average 14.3 observation per patient
- ⁴ n= 90 with average 14.4 observation per patient