Prognostic factors and the response to hydroxyurea treatment in sickle cell disease

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Abstract

This review describes current considerations in the use of hydroxyurea for the management of sickle cell disease in the context of clinical severity. Randomized trials of hydroxyurea have generally enrolled subjects with increased severity based on frequent vaso-occlusive events. An exception was the BABY HUG study in infants which documented substantial benefit even for asymptomatic subjects. Increasing data indicate that hydroxyurea has a substantial effect on reducing mortality in both adults and children—perhaps the most compelling reason for advocating the drug's widespread use. Although the efficacy of hydroxyurea is mediated primarily through increased erythrocyte fetal hemoglobin and much has been learned about the genomic influences on fetal hemoglobin levels in sickle cell disease, our ability to predict the fetal hemoglobin response to hydroxyurea remains limited; much more work in this area is indicated. The review is concluded with the recommendations of the 2014 NIH Evidence-Based Management of Sickle Cell Disease Expert Panel Report.

Keywords: Sickle cell, hydroxyurea, clinical severity, mortality, pharmacogenetics, prognosis

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Introduction

A particular goal of this issue of the journal is to describe our understanding of the clinical severity of sickle cell disease (SCD). Classification by severity in SCD has been attempted through a variety of formulas involving both clinical and laboratory parameters. In the 1980s and 90s, the Cooperative Study of Sickle Cell Disease (CSSCD) collected data on more than 4000 subjects with SCD to document the “natural history” of the condition. The CSSCD utilized a standardized definition for vaso-occlusive pain crisis, in which hospitalization or an emergency department visit were required, and reported the annual frequency of these events in a large cohort of patients with HbSS; 5% of patients had 3–10 events per year.¹ A higher rate of pain events was associated with higher mortality (in patients over age 20 years) and lower HbF levels. Subsequently, an analysis of the “infant cohort” from the CSSCD concluded that an episode of dactylitis before age 1 year, an Hb level of <7 gm/dL, and leukocytosis were correlated with a more severe subsequent course, including early mortality.² However, in the Dallas Newborn SCD Cohort, which included patients followed between 1983 and 2007, mortality during childhood was much lower (~5% overall), largely due to a marked reduction in the incidence of pneumococcal sepsis in early childhood, resulting from the use of penicillin prophylaxis and pneumococcal vaccines.³ However, no

widely accepted definition of clinical severity in SCD is currently available, a major limitation in evaluating the efficacy and effectiveness of hydroxyurea (HU) therapy and a barrier to making comparisons among different clinical trials.

In the United States, HU is currently the only drug approved by the Food and Drug Administration (FDA) for treatment of SCD with an indication in severely affected adults. Despite restricted indications, HU is the most common intervention utilized in the long-term management of SCD; more than 50% of patients with HbSS are treated with the drug in many pediatric sickle cell centers, although adherence may be problematic.⁴ Recent articles have reviewed the mechanisms of action of HU and provided comprehensive updates of the clinical effects of the drug.^{5,6} However, factors derived from clinical history or laboratory evaluation which can predict the response to HU treatment are lacking. This review will summarize current understanding of prognostic factors related to response to the drug, including the predictive value of the severity of the patient's clinical condition.

Fetal hemoglobin and HU

Fetal hemoglobin (HbF) inhibits the polymerization of deoxyhemoglobin S because neither HbF ($\alpha_2\gamma_2$) nor the

hybrid tetramer $\alpha_2\gamma\beta^S$ is incorporated into the polymer.⁷ In African-Americans with sickle cell anemia (HbSS), there is stabilization of HbF levels by about age 4 years, resulting in baseline values that average 8–10% (range ~3–20%).⁸ The effect of HbF in ameliorating the clinical and hematologic complications of SCD has been recognized since the 1950s. Initial observations included: (i) the delayed onset of manifestations of SCD until after the first 4–6 months of life when HbF declines to low levels; (ii) the protection offered by inheritance of a hereditary persistence of fetal hemoglobin (HPFH) gene in combination with the gene for HbS; and (iii) the relatively mild symptomatology of subjects from central India and eastern Saudi Arabia who have high HbF levels. Subsequently, data from the CSSCD demonstrated that higher HbF was associated with diminished rates of pain events, acute chest syndrome (ACS), and mortality.^{1,9}

HU is a ribonucleotide reductase inhibitor that has been used to treat myeloproliferative disorders such as chronic myeloid leukemia (CML) since the 1960s.¹⁰ However, in 1984, reports that both anemic monkeys and sickle cell patients had increased HbF production when treated with HU^{11,12} led to intense research in the clinical use of the drug. The exact mechanisms by which HU induces HbF production are not fully understood. Cytotoxic effects of HU on the bone marrow produce stress erythropoiesis with increased HbF production.¹³ *In vitro* studies utilizing K562 erythroleukemia cells and human erythroid progenitor cells have demonstrated that HU *nitrosylates* and activates soluble guanylate cyclase (SGC) with subsequent increased expression of cyclic GMP (cGMP)-dependent protein kinase.^{14,15} The nitric oxide (NO)-SGC-cGMP pathways are thought to play a role in induced expression of γ -globin.

HU has a number of beneficial hematologic and biochemical effects related to or in addition to induction of HbF, including prolonged erythrocyte lifespan (decreased hemolysis), decreased neutrophil and reticulocyte counts resulting from marrow cytotoxicity, reduced expression of adhesion molecules on neutrophils and red cells, increased erythrocyte hydration and deformability, and local release of NO, potentially resulting in vasodilatation.^{16–18} Another mechanism was suggested from a study of HU in sickle cell

mice, in which survival after pneumococcal pneumonia was improved by reduction of their abnormally elevated inflammatory response.¹⁹ A reduction in adenosine activity in HU-treated patients may also be of value.²⁰

HU and eligibility for clinical trials

Only four randomized placebo-controlled trials of the use of HU in sickle cell anemia (SCA) have been published.^{21–24} The eligibility criteria for these studies and one important multicenter non-randomized phase I–II pediatric study²⁵ are summarized in Table 1. The landmark double-blinded phase III trial which led to FDA approval of the drug for adults with clinically severe SCA was the Multicenter Study of Hydroxyurea (MSH), in which 299 patients were randomized to receive HU or placebo.²¹ The study was halted early due to a statistically significant increase in the time to the first painful vaso-occlusive event in the HU arm. Persons randomized to this arm had 40–50% reduction in the incidence of painful events, ACS, transfusions, and hospitalizations. The first randomized trial in children was a single-blind crossover study in which patients received six months of HU and six months of placebo. The 22 evaluable patients demonstrated reduction in the number of days in the hospital and the length of hospital stay when receiving HU.²² The largest of several non-randomized studies in children with SCA in the U.S. was HUG-KIDS, a phase I–II trial in 84 school age children with SCA,²⁵ who showed similar hematologic responses to those seen in adults, including increases in HbF, MCV, and hemoglobin levels and decreases in WBC and absolute neutrophil counts. More recently, the phase III double-blinded multicenter BABY HUG trial²³ involved 193 infants between 9 and 18 months of age, who were randomized to receive HU (at a fixed dose of 20 mg/kg/day) or placebo for a two-year period, with the primary objectives of demonstrating better preservation of organ function in the spleen (based on uptake on Tc⁹⁹ liver-spleen scans) and kidneys (based on glomerular filtration rate measured by Tc⁹⁹DTPA renal clearance). Although the primary endpoints were not achieved, the study demonstrated that the HU group had fewer episodes of pain, dactylitis, ACS, hospitalizations and

Table 1 Eligibility for randomized placebo-controlled hydroxyurea trials

Trial	Population	Eligibility (Dx)	Eligibility (severity)
Charache et al. ²¹	Adults (≥ 18 year old)	HbSS	≥ 3 crises reported to physician in year before entry
Ferster et al. ²²	Children (2–22 year old, med. = 9 year old)	HbSS in Belgium	> 3 VOC in previous year or Hx of stroke, ACS or splenic sequestration
Kinney et al. ^{25,a}	Children (5–15 year old, med. = 9.8 year old)	HbSS	≥ 3 pain events in previous year ≥ 3 episodes of ACS in previous two years three episodes of pain or ACS in previous year
Wang et al. ²³	Infants (9–18 months old, med. = 13 months old)	HbSS/S β^0 thal	None
Jain ^{24,b}	Children (< 18 year old)	HbSS in India	> 3 episodes of VOC or transfusions/year

ACS: acute chest syndrome; VOC: vaso-occlusive crisis.

^aNot randomized placebo-controlled trial.

^bTrial of low-dose HU.

transfusions, along with higher levels of hemoglobin, HbF, and MCV, and decreased WBC, neutrophil, and reticulocyte counts. Administering daily HU to this young population using a liquid formulation was feasible and well tolerated. There was no significant toxicity, except for the expected reversible mild to moderate neutropenia, and this was not associated with increased risk for invasive infection. Most patients from the BABY HUG trial continue in a long-term follow-up study, which will be crucial for identifying long-term benefits and risks. One other recent randomized trial involved children with SCD from India, who were randomized to a fixed low dose of HU or placebo. Those receiving HU had increased Hb and HbF levels and fewer pain episodes, transfusions, and hospitalizations.²⁴

These studies were similar in their eligibility criteria except for the BABY HUG trial (Table 1). In general, they involved an above average level of severity, characterized by a least three vaso-occlusive events (pain crises or episodes of ACS) in the year prior to participation in the study. Numerous single institution reports of the response to HU have used similar criteria for initiation of treatment.^{26,27} The BABY HUG trial was unique in not requiring evidence of increased clinical severity for eligibility.²³ To be enrolled in this study, subjects had to have a diagnosis of HbSS or HbS β^0 thalassemia and be 9–18 months of age. They were excluded if they had height, weight, or head circumference less than the 5th percentile; mental development index less than 70; or abnormal transcranial Doppler ultrasound (TCD) velocity.

Efficacy of HU in asymptomatic young children

To examine the efficacy of HU in young children who had had no clinical events related to SCD prior to participation in the BABY HUG trial, the subsets of asymptomatic patients randomized to HU ($n=52$) and to placebo ($n=49$) were compared²⁸ (Table 2). Statistically significant differences ($P=0.006$ – 0.04) and increased hazard ratios

were seen in the placebo group for dactylitis, hospitalizations, and transfusions – findings similar to those reported for the study as a whole. Although “pain alone” and ACS occurred more often in the placebo group, differences did not reach statistical significance ($P=0.14$ and 0.17 , respectively). In general, the benefit of HU in reducing the number and frequency of sickle cell-related complications in asymptomatic infants mirrored that seen in clinically severe adults and older children and in the BABY HUG cohort as a whole.

HU and mortality

Data from the CSSCD, published 25 years ago, showed that increased vaso-occlusive pain events were associated with increased mortality.¹ More recent data have confirmed this association and have also found associations with elevated WBC count, renal dysfunction (decreased GFR, proteinuria), pulmonary hypertension (elevated tricuspid regurgitant jet velocity), and cerebrovascular events.^{29–31} A major advantage of HU has been its limited toxicity, which has not included any fatalities directly attributable to the drug. Of note, one case of fatal splenic sequestration occurred in an infant on HU on the HUSOFT protocol (among 28 treated),²⁷ and no deaths occurred among the 95 who received HU in the BABY HUG trial.²³

Although the benefits of HU on vaso-occlusive complications and laboratory parameters are impressive, the most compelling rationale for its use may be its effect on mortality. Five reports have compared patients who received HU treatment with those who did not (or who received suboptimal or shorter length treatment)^{32–36} (Table 3). Three reports involved adults with SCD. In a 17.5 year follow-up of the 299 original patients from the MSH study, mortality was significantly reduced in individuals with long-term HU exposure.³² In the Greek LaSHS study of 330 adult patients with HbSS, S β^0 - or S β^+ -thalassemia, significant reductions in pain, ACS, transfusion, hospitalization, stroke, and overall mortality occurred with HU (10-year survival in the HU-treated group 86% versus 65% in those untreated).³³

Table 2 Clinical events in children who were asymptomatic at enrollment in the BABY HUG Trial^a

	Hydroxyurea, N = 52, patient-year = 100		Placebo, N = 49, patient-year = 96		Analysis	
	Events	Subjects	Events	Subjects	Hazard ratio (95% CI) ^b	P ^a
Pain (all events)	61	30	128	31	1.3 (0.8, 2.1)	.35
Pain alone	31	17	46	24	1.6 (0.9, 3.0)	.14
Dactylitis	6	4	35	14	4.2 (1.4, 12.9)	.008
ACS	3	3	10	7	2.5 (0.7, 9.7)	.17
Hospitalization	80	31	149	43	1.9 (1.2, 3.0)	.006
Transfusion	11	6	27	14	2.7 (1.0, 6.9)	.04
Splenomegaly	37	12	42	17	1.6 (0.7, 3.3)	.24
Splenic sequestration	3	2	10	7	3.8 (0.8, 18.4)	.07
Sepsis	0	0	3	3		

ACS: acute chest syndrome; CI: confidence interval.

^aThornburg et.al.²⁸

^bThe hazard ratio (hydroxyurea vs. placebo) and 95% CI were generated from a Cox model and the P value from the log-rank life test to compare the time to first event between the two treatment groups.

Table 3 Hydroxyurea and mortality

Authors/location	Pt. population/ study design	Genotype/inclusion criteria	N	Follow-up periodmean/ median(range)	Hospital use	Mortality
Steinberg et al. ³² (MSH)/ North America	Adults/ prospective	SCA; MSH cohort; analysis by HU use, not original trial assignment	129/299 of original cohort deceased	Up to 17.5 y		HU for at least 5 y (compared with <5 y) had ↓ mortality
Voskaridou et al. ³³ (LaSHS)/Greece	Adults/ prospective	HbSS: 34, HbSβ ⁺ thal: 131, HbSβ ⁺ thal: 165; ≥3 VOC/pre- vious y; CVA or ACS in past 5 y	330 (HU:131 [SCA:87; Sβ ⁺ thal:44])	HU: 8 y (0.1–17) No HU: 5 y (0.1–18)	↓; <i>P</i> < .001	10-y survival: 86% for HU; 65% for non-HU; <i>P</i> = .001
Lobo et al. ³⁴ / Brazil	Children/ retrospective	SCD; indications for HU: recurrent VOC; >1 ACS; Hb <6 g/dL; CVA	1760 (HU:267)	7 y (3–17) HU: 2 y (0.1–6.5)	↓50%; <i>P</i> < .001 ED visits ↓35% <i>P</i> < .001	↓ mortality; 1 death in HU group; 37 in no-HU group
Lê et al. ³⁵ / Belgium	Adults + children/ retrospective	SCD	At last F/U: 185-HU 170-no rx 90-HSCT	10.3 y (0.1–49)		HU = 3 (1.6%) No rx = 5 (2.9%), HSCT = 5 (5.6%)
Fitzhugh et al. ³⁶ /U.S.A.	Adults/ retrospective	HbSS	383 (good dose HU: 156; no HU: 130)	~6 y		HU → improved survival (hazard ratio = 0.58, <i>P</i> = 0.04)

HU: hydroxyurea; SCD: sickle cell disease; SCA: sickle cell anemia; HSCT: hematopoietic stem cell transplantation; ACS: acute chest syndrome; MSH: multicenter study of hydroxyurea.

In a recent review of 383 adults with HbSS from the NIH, there were 59 deaths, which were significantly related to hepatic, kidney, and cardiopulmonary dysfunction.³⁶ HU use was associated with improved survival, particularly in those taking the recommended dose of 15–35 mg/kg/d (hazard ratio 0.36, *P* = 0.005).

Two reports of the effects of HU on mortality have involved children. In a study from Rio de Janeiro, Brazil, among 1760 children with SCD, 267 clinically severe patients were treated with HU for a median of two years.³⁵ Although this was based on retrospective data and not a randomized trial, the HU-treated children had significant reductions in hospitalizations, transfusions, and, most notably, mortality, due to fewer deaths from ACS and infection (99.5% vs. 94.5% survival, *P* = 0.01). In another recent report, data from the Belgian SCD Registry covering 469 pediatric and adult patients and 5110 patient-years of follow-up were reviewed.³⁵ These subjects included 185 who were receiving HU at last follow-up, 90 who had received hematopoietic stem cell transplantation (HSCT), and 170 who had never had therapeutic intervention. HU patients had a lower mortality rate compared with those receiving HSCT and those with no intervention (0.14, 0.36, and 0.38 per 100 patient-years, respectively) as well as higher Kaplan–Meyer estimates of 15-year survival (99.4%, 93.8%, and 95.4%, respectively).

Although these reports were retrospective, the cohorts receiving HU had more severe disease (generally based on vaso-occlusive events), and therefore a worse prognosis. However, all five studies found a highly significant survival

advantage for those who had been treated with therapeutically appropriate HU. Although these results, which were consistent in both pediatric and adult populations, do not directly address the question of whether HU is advantageous for patients who have had a mild clinical course, they demonstrate that those with a more severe clinical course have better survival on HU.

Predicting the response to HU

Although the NHLBI Evidence-Based Panel (see below) has recommended that HU be offered to asymptomatic infants with SCA, most reports of the drug's efficacy have involved children or adults with clinically "severe" disease. In the limited number of randomized trials described above, the subjects receiving HU or placebo were balanced at randomization so that the cohorts were composed of patients of equivalent severity. Following completion of the MSH and the HUG-KIDS trial, potential prognostic factors for response to HU, usually defined as the HbF level (or change in HbF level) at maximum tolerated dose (MTD) of HU, were examined. These data provide some insight into the question of how baseline clinical severity may influence response to the drug.

In the MSH trial in symptomatic adults, the pretreatment vaso-occlusive crisis (VOC) rate was, not surprisingly, associated with the rate on treatment.²¹ Gender, age, α -globin status, and β -globin haplotype were not predictive of VOC response nor were baseline clinical laboratory values. In a later analysis, increased baseline absolute neutrophil count (ANC) and absolute reticulocyte count (ARC), but not HbF,

were associated with greater HbF response.³⁷ Of note, those in whom plasma samples showed the presence of HU less than 50% of the time had a greater VOC rate, indicating that adherence with taking the drug was probably an important determinant of response. In the HUG-KIDS study, baseline HbF (unlike the MSH), Hb level, ARC, and WBC count were associated with the HbF level at MTD, as were the increases in Hb level and MCV and decreases in ARC and WBC.³⁸ MTD dose and compliance with taking HU were also associated with the HbF level reached. However, baseline clinical severity was not analyzed as a predictor. Thus, neither MSH nor HUG-KIDS addressed the prognostic significance of baseline clinical severity, nor did several local or regional long-term follow-up studies.^{39,40}

Pharmacokinetics, pharmacogenetics, and response to HU

Currently in the U.S., HU is approved by the FDA only in capsular form for adults with “severe” SCA. A liquid formulation with HU dissolved in simple syrup at a concentration of 100 mg/mL is commonly used in younger children,⁴¹ but FDA review is still pending. Pharmacokinetics (PK) evaluation of HU has been limited. In a French study, PK profiles in 11 children given a breakable HU tablet demonstrated a C_{max} of 24.5 μ g/mL and a T_{max} of 0.75 h.⁴² In another study, PK testing following a first dose of liquid HU showed a C_{max} of 25.4 μ g/mL and a $T_{1/2}$ of 1.6 h; both slow and fast concentration-time profiles were seen.⁴³ Recently, in children with SCD, a solution of HU in cherry syrup was compared with HU in capsules and showed bioequivalence, indicating that dosing adjustments for the different drug formulations were unnecessary.⁴⁴ Although little is known about mechanisms that produce variability in HU PK, organic anion transporting polypeptide 1b (OATP1b) transporters have recently been shown to modulate HU PK.⁴⁵

Since HbF levels have been recognized as the most closely associated laboratory marker of clinical prognosis in SCD for decades, much of recent molecular research has targeted genetic influences on levels of this hemoglobin and the switch from γ -globin to β -globin production. This has led to impressive progress in identification of genes associated with the determination of HbF levels, primarily *BCL11A* and the intergenic interval between *HBS1L* and *MYB* (*HBS1L-MYB*).⁴⁶ *Xmn1* polymorphism was thought to explain 2–10% of HbF variation, but recent evidence suggests that this site is not the functional variant.⁴⁷

However, the dramatic increase in understanding the molecular mechanisms of HbF production has not translated into significant progress in predicting the HbF response to HU treatment. Previously, in the HUG-KIDS trial, the strongest predictor of HbF at MTD was the baseline HbF level.³⁸ More recently, HbF at MTD was again found to be associated with baseline HbF, but pharmacogenetic (PG) analysis did not reveal single nucleotide polymorphisms (SNPs) influencing HbF at MTD.⁴³ In another study, baseline HbF explained 33% of the induced level of HbF, but a variant in the ϵ -globin locus, which added an additional 13%, was the only other contributor to

the variance.⁴⁸ In other recent studies, the Bantu haplotype conferred a greater response to HU treatment,⁴⁹ a homozygous mutant state of a KLF10 SNP was associated with a poor response to HU,⁵⁰ and a coding variant in *SALL2* (Spalt-like transcription factor), identified through whole exome sequencing, was associated with a higher final HbF.⁵¹

In an ancillary analysis from the BABY HUG trial, alpha thalassemia, beta-globin haplotype, and polymorphisms known or presumed to affect HbF levels (*BCL11A*, *HBS1L-MYB*, *Xmn1*) were studied in 190 randomized subjects.⁵² At study entry, infants with alpha thalassemia trait had lower MCV, bilirubin and reticulocyte counts; beta-globin haplotypes containing the SEN (Senegal) polymorphism were associated with higher Hb and HbF levels; and *BCL11A* and *Xmn1* polymorphisms affected baseline HbF. At study exit, subjects randomized to HU had drug treatment effects that superseded most genetic influences. Neither *BCL11A* nor *HBS1L-MYB* polymorphisms or β -globin haplotype influenced the degree of HbF induction by HU. Thus, in general, molecular determinants of baseline HbF level have been identified, but PG predictors of HbF response to HU have not been found. Genetic studies of larger populations on HU and of unusual responders, and the influence of specific sequence variants, ideally analyzed with whole genome sequencing, are needed to better predict HbF response to the drug.⁵ Nevertheless, the rapidly increasing understanding of molecular mechanisms associated with γ - to β -globin switching, such as identification of transcription factors which downregulate the function of the *BCL11A* gene, has led to targets for gene editing approaches currently underway for ameliorating SCD.^{47,53}

Recommendations for HU use in SCD

In 2008, a consensus conference at the NIH concluded that HU is efficacious in both children and adults with SCA, and that it is greatly underutilized.⁵⁴ Subsequently, data from the BABY HUG trial showed a similar or even greater degree of efficacy of HU, when compared to that seen in the MSH trial in adults, in reducing the morbidity of the most common vaso-occlusive complications of SCD.²³ An important distinction between the MSH and BABY HUG trials is that the latter involved subjects who were *not selected on the basis of clinical severity*. HU has been well tolerated in both adults and children. Although important data still need to be collected, more than two decades of long-term follow-up have not revealed major safety concerns associated with the drug.

In 2014, an NHLBI Expert Panel published Evidence Based Guidelines for the management of SCD, recommending that adults with SCA who have ≥ 3 moderate to severe pain crises during a 12-month period, sickle cell-associated pain or severe symptomatic chronic anemia that interferes with daily activities and quality of life, or a history of severe or recurrent ACS should be treated with HU.⁵⁵ These were strong recommendations based on moderate to high-quality evidence. The recommendations were even broader in children: In infants nine months or older, in children, and in adolescents with SCA, treatment with HU should be offered

regardless of clinical severity to reduce complications (e.g. pain, dactylitis, ACS, anemia) related to SCD. These were considered to be strong recommendations for children, based on high-quality evidence for patients aged 9 to 42 months. The NHLBI Panel recommended that HU treatment should be offered as a mechanism to open discussion with families about the risks and benefits of HU therapy in asymptomatic infants. In addition, the panel recommended that persons with HbS β^+ -thalassemia or HbSC who had recurrent sickle cell-associated pain consult a "sickle cell expert" for consideration of HU therapy. The Guidelines also included a detailed treatment protocol for implementation of HU therapy. Although the Guidelines were based on the extensive clinical and hematologic benefits of HU, particularly the evidence from the MSH and BABY HUG studies, the decreased mortality associated with HU treatment may now be the most compelling argument for treating all SCA patients, regardless of severity.

DECLARATION OF CONFLICTING INTERESTS

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