CLINICAL OBSERVATIONS, INTERVENTIONS, AND THERAPEUTIC TRIALS

Safety of Hydroxyurea in Children With Sickle Cell Anemia: Results of the HUG-KIDS Study, a Phase I/II Trial

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Previous studies have determined the short-term toxicity profile, laboratory changes, and clinical efficacy associated with hydroxyurea (HU) therapy in adults with sickle cell anemia. The safety and efficacy of this agent in pediatric patients with sickle cell anemia has not been determined. Children with sickle cell anemia, age 5 to 15 years, were eligible for this multicenter Phase I/II trial. HU was started at 15 mg/kg/d and escalated to 30 mg/kg/d unless the patient experienced laboratory toxicity. Patients were monitored by 2-week visits to assess compliance, toxicity, clinical adverse events, growth parameters, and laboratory efficacy associated with HU treatment. Eighty-four children were enrolled between December 1994 and March 1996. Sixty-eight children reached maximum tolerated dose (MTD) and 52 were treated at MTD for 1 year. Significant hematologic changes included increases in hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, and fetal hemoglobin parameters, and decreases in white blood cell, neutrophil, platelet, and reticulocyte counts. Laboratory toxicities typically were mild, transient, and were reversible upon temporary discontinuation of HU. No life-threatening clinical adverse events occurred and no child experienced growth failure. This Phase I/II trial shows that HU therapy is safe for children with sickle cell anemia when treatment was directed by a pediatric hematologist. HU in children induces similar laboratory changes as in adults. Phase III trials to determine if HU can prevent chronic organ damage in children with sickle cell anemia are warranted.
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HYDROXYUREA (HU) is effective therapy for adults with sickle cell anemia. In a controlled trial, patients receiving HU had a lower rate of painful events, acute chest syndrome, need for transfusion, and hospitalization compared with patients taking placebo.[1] The principal toxicities observed in this study were transient reversible depressions of white blood cells (WBCs), platelets, and hemoglobin concentration.

Several small studies have reported the short-term toxicity and efficacy of HU for children with sickle cell anemia.[2-6] There is appropriate reluctance, however, to use this drug in children because of concerns related to potential toxicities including impaired growth, teratogenesis, or carcinogenesis.[7,8] These concerns have hindered implementation of clinical trials to test the efficacy of HU in children.

This report describes the results of a multicenter Phase I/II trial of HU in children with sickle cell anemia (HUG-KIDS). The study was designed to determine the maximum tolerated dose (MTD) of HU and to treat a cohort of 50 severely affected children for 1 year at MTD. The specific aims were to determine if: (1) HU will elevate the fetal hemoglobin (Hb F) level, hemoglobin concentration, and red blood cell mean corpuscular volume (MCV) above baseline values; (2) hematologic and other toxicities of HU are similar to those in adults; (3) HU therapy has an adverse effect on growth.

MATERIALS AND METHODS

Patient selection. Children with severe sickle cell anemia, ages 5 to 15 years, were eligible for enrollment. Severe disease was defined as 3 or more pain events (PEs) within the year before entry, or at least 3 episodes of acute chest syndrome (ACS) requiring hospital admission within 2 years of entry, or any combination of 3 episodes of ACS or PEs within 1 year of the enrollment. Eligible patients also needed a minimum of 6 documented height and weight measurements recorded for at least 2 years preceding entry.

Exclusion criteria included pregnancy, seropositivity for human immunodeficiency virus, or another chronic illness that potentially could enhance HU toxicity. Patients also were excluded if they were taking medications on a regular basis that might enhance HU toxicity, such as theophylline, estrogen, or calcium channel blockers. Other exclusion criteria included prior treatment with HU, a serum creatinine greater than 1.0 mg/dL, serum alanine aminotransferase (ALT) more than twice the upper limit of normal, a red blood cell transfusion within 100 days of enrollment, or more than 10% Hb A on hemoglobin electrophoresis at the time of entry.

Enrollment was complete once informed consent had been obtained in accordance with the guidelines of each participating center's Institutional Review Board, the enrollment criteria had been reviewed by the Study Coordinator (EEO), and the intake form had been received by the Statistical Center. Intake laboratory tests included a complete blood count, differential white count, absolute reticulocyte count (ARC), urinalysis, hemoglobin electrophoresis with measurements of

Hb A, Hb A$_2$ and Hb F, F-cell quantitation, serum levels for iron and total iron binding capacity, ferritin, B$_{12}$, folate, hepatitis B surface antigen, and a serum chemistry panel. This panel included creatinine, total protein, albumin, lactic acid dehydrogenase (LDH), aspartate aminotransferase (AST) and ALT, and total bilirubin concentration. Other intake laboratory tests included measurement of serum antibodies to hepatitis A, hepatitis B, hepatitis C, and Parvovirus B19. Pregnancy tests were performed on menstruating patients before initiating HU treatment.

Patient monitoring. Interval histories were obtained at biweekly intervals. A complete physical examination was performed every 4 weeks. Laboratory monitoring included a complete blood count with differential every 2 weeks, a serum chemistry panel every 4 weeks, Hb F parameters every 8 weeks, and an assessment of iron stores every 6 months. Pregnancy tests were performed if menses were delayed by more than 2 weeks.

Interval histories, physical examinations, and laboratory results were faxed to the Statistical Center. Values for hemoglobin concentration, ARC, absolute neutrophil count (ANC), platelet count, serum creatinine, and serum ALT were checked immediately for toxicity at the participating center and again on receipt at the Coordinating Center.

To reduce the risk of toxicity, only a 2-week supply of HU was dispensed at a time. Patients were requested to return all unused HU at each study visit. A Drug Safety Monitoring Board (DSMB) was appointed by the National Institutes of Health to oversee the HUG-KIDS trial.

Dosing schedule. Patients initially were prescribed 15 mg/kg of HU orally as a single daily dose. The daily dose was increased by 5 mg/kg every 8 weeks in the absence of toxicity. If a patient experienced a laboratory toxicity, HU was discontinued for at least 1 week. Once the toxicity resolved, HU was restarted at a dose 2.5 mg/kg lower than the dose at which the toxicity occurred. HU doses then were increased subsequently in 2.5 mg/kg increments every 8 weeks, provided toxicity did not occur. MTD was defined as the dose 2.5 mg/kg below which 2 successive hematologic toxicities occurred or when the daily dose reached 30 mg/kg and was sustained without toxicity for 8 weeks. Once MTD was achieved, patients were treated for 1 year and then exited from the study.

Laboratory methods. Blood counts, serum iron studies, and serum chemistries were performed by standard techniques at each clinical center. The fetal hemoglobin parameters (Hb F level and F-cell percentage) were measured at the Children's Hospital of Philadelphia. The Hb F level was determined by high-pressure liquid chromatography and F cells were assayed by an immunofluorescence method.[9-11]

Definition of toxicities, adverse events, and growth failure. Toxicities were assigned to 1 of 3 groups: hematologic, hepatic, or renal. Hematologic toxicity was defined as 1 or more of the following: ANC below 2.0×10^9/L; an ARC below 80×10^9/L unless the hemoglobin concentration was 9.0 g/dL or higher; a platelet count below 80×10^9/L; a 20% decrease in hemoglobin concentration from entry or previous value, or a hemoglobin concentration below 4.5 g/dL. Hepatic toxicity was defined as an ALT value that was greater than twice the upper limit of normal. A 50% increase from baseline in the serum creatinine and a value of more than 1.0 mg/dL defined renal toxicity.

An adverse event (AE) was defined as death or any life-threatening or clinical event likely to interfere either temporarily or permanently with the patient's ability to continue or tolerate HU therapy. Growth failure was defined as a growth velocity less than the fifth percentile for age over a 6-month period.

Study coordination, data management, and statistical methods. The Study Coordinator monitored all clinical aspects of this study. Staff at the Statistical Center managed all data and correspondence. To ensure confidentiality, patients were identified only by an acrostic and unique patient identification number.

Clinic sites faxed forms to both the Study Coordinator and the Statistical Center. When received at the Statistical Center, these images were stored in a RhoFAX image database (Rho, Inc, Chapel Hill, NC). Before data entry, the Study Coordinator and Statistical Center personnel reviewed forms for content, accuracy, and completeness. The study's database management system used independent double data entry with a third party referee, followed by field-specific (valid value, valid range) and multivariate error detection procedures. Questionable values were faxed to each site's coordinator and responses were faxed back to the Statistical Center.

Statistical analyses were performed with SAS software (Cary, NC). The Student's *t*-test was used to compare means between groups.

RESULTS

Enrollment. Eighty-four African-American children with sickle cell anemia were enrolled from December 14, 1994 through March 31, 1996. The patient population included 40 females, 7 of whom had attained menarche before enrollment. The mean patient age ($\pm$1 standard deviation [SD]) was 9.8 $\pm$ 3.2 years, with a median age of 9.1 years and a range from 5 years to 15 years. Three children, not included in the 84, were enrolled, but never received HU. One of these patients was not homozygous for the sickle hemoglobin gene and legal guardians of the others withdrew their children before administering HU.

HU compliance. Capsule counts were analyzed as a surrogate for compliance. Patients were expected to have taken all previously dispensed HU capsules when they returned to the clinic at each 2-week interval. Of 3,393 medication refill visits, 74% were reported as having no pills returned. Capsules were returned at 26% of the visits, including 10% of visits with $\leq$10% of pills returned and 10% of visits where 10% to 25% of pills returned. Only 6% of visits had more than 25% of pills returned.

Laboratory effects of HU therapy. Changes in laboratory values are summarized in Table 1. By 6 months of HU treatment, there were statistically significant increases in the hemoglobin concentration, MCV, mean corpuscular hemoglobin (MCH), Hb F level, and percentage of F cells and significant decreases in the ARC, WBC count, ANC, platelet count, total bilirubin, and LDH compared with baseline values ($P < .0001$). The mean corpuscular hemoglobin concentration (MCHC), ALT, and creatinine were not significantly different from baseline values at 6 months.

After 12 months of HU treatment, there was significant difference ($P < .05$) in the mean MCV, MCH, LDH, total bilirubin, Hb F, and F-cell percentage compared with 6 months. There were no significant changes in the ALT or creatinine after 12 months of treatment compared with their respective baseline or 6-month values.

MTD. When this trial ended, 68 patients had attained MTD, 52 of the 84 enrolled patients (62%) had completed 1 year of HU treatment at MTD, 10 patients were still being treated at MTD, and 4 patients were in the dose-escalation phase.

By protocol design, the minimum time required to attain an MTD of 30 mg/kg was 224 days. The average time for patients to reach MTD was 330 $\pm$ 164 days with a median of 263 days. For the 68 patients who achieved MTD, the mean HU dose was 25.6 $\pm$ 6.2 mg/kg, while the median MTD was 30 mg/kg with a range from 7.5 to 30 mg/kg.

Table 1. Laboratory Parameters (mean ± standard deviation) During HU Therapy

Variable	Entry	6 mo	12 mo	18 mo	24 mo
No. of patients	84	78	77	71	35
Hematology					
Hb (g/dL)	7.8 ± 1.0	8.8 ± 1.0	9.0 ± 1.4	9.1 ± 1.3	9.0 ± 1.1
MCV (fL)	85.9 ± 6.6	99.5 ± 9.0	101.3 ± 10.2	101.7 ± 10.5	98.9 ± 9.1
MCH (pg)	29.5 ± 2.8	34.1 ± 3.4	34.6 ± 3.6	34.5 ± 3.8	33.9 ± 3.1
MCHC (g/dL)	34.3 ± 1.6	34.2 ± 1.1	34.2 ± 1.4	34.0 ± 1.0	34.3 ± 1.5
ARC (×10⁹/L)	354 ± 144	204 ± 83	191 ± 100	200 ± 82	215 ± 92
WBC (×10⁹/L)	13.6 ± 3.9	9.3 ± 3.0	9.2 ± 3.2	9.1 ± 3.1	9.2 ± 3.0
ANC (×10⁹/L)	7.0 ± 3.0	4.4 ± 2.1	4.4 ± 2.2	4.4 ± 2.6	4.6 ± 2.3
PLT (×10⁹/L)	461 ± 157	371 ± 130	371 ± 153	357 ± 144	357 ± 122
Serum chemistries					
Total bilirubin (mg/dL)	3.6 ± 2.6	2.9 ± 2.1	2.5 ± 2.0	2.5 ± 1.8	2.8 ± 2.5
LDH (IU/L)	1,126 ± 699	921 ± 592	807 ± 520	858 ± 490	973 ± 500
ALT (IU/L)	27 ± 14	28 ± 20	28 ± 22	26 ± 13	27 ± 12
Creatinine (mg/dL)	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.1
Hb F parameters					
Hb F (%)	7.3 ± 4.9	14.9 ± 6.4	17.8 ± 7.2	16.3 ± 7.2	15.5 ± 7.3
	(N = 69)	(N = 70)	(N = 69)	(N = 62)	(N = 31)
F cells (%)	34.6 ± 17.8	59.2 ± 18.6	66.5 ± 19.6	62.9 ± 18.1	62.2 ± 18.1
	(N = 72)	(N = 72)	(N = 68)	(N = 66)	(N = 33)

All values at 6 months, except MCHC, ALT, and creatinine are significantly different from baseline ($P < .0001$). At 12 months, MCV, MCH, LDH, and total bilirubin are significantly different compared with 6 months ($P < .05$) and the Hb F and F cells are significantly different at the level of $P < .05$. All other 12-month values are not significantly different than their corresponding 6-month values.

Patient attrition. Eighteen patients were removed from the study, including 6 patients who had achieved MTD. These included 10 patients who were noncompliant with HU administration or unable to meet the required visit schedule and 3 patients who relocated away from a clinical center. The DSMB requested that 4 patients be removed from the study and 1 other patient had HU discontinued by the study physician because of sickle cell–related complications.

The first patient withdrawn by the DSMB had received an unsuccessful bone marrow transplant before enrollment and experienced recurrent thrombocytopenia while receiving HU. Another patient was removed because of recurrent elevations of the serum ALT. Review of this patient's medical records showed a long history of intermittent ALT elevations before enrollment that were not associated with specific hepatobiliary complications or clinical events. The third patient complained of severe recurrent headaches during HU therapy. Cerebral arteriography demonstrated vasculopathy with a moya-moya pattern. The patient was placed on a chronic transfusion program. The last patient had transient aphasia and left-sided weakness immediately after erythrocytapheresis in preparation for an elective cholecystectomy. His postapheresis hemoglobin concentration was 13.8 g/dL and the transient ischemic attack was attributed to hyperviscosity.

The only patient removed by a study physician was a child with documented hypersplenism and recurrent neutropenia for 18 months before HU therapy. HU was stopped because the patient remained neutropenic while receiving a very low dose of HU (2.5 mg/kg/d).

Laboratory toxicity. Cytopenias were the most common side effect from HU therapy, as would be anticipated in a Phase I/II trial of a myelosuppressive agent designed to achieve the MTD (Table 2). Neutropenia was the most common hematologic toxicity, but only occurred in 5.2% of all blood count measurements. The majority of neutropenia toxicities were mild with an ANC value between 1.5 and 2.0×10^9/L. No episode of neutropenia was associated with a serious infection. Reticulocytopenia and anemia also occurred, but much less frequently than did neutropenia. Anemia severe enough to warrant transfusion was observed only in association with episodes of ACS. No patient was transfused for severe anemia solely due to the myelosuppressive effect of HU. One patient experienced an episode of severe thrombocytopenia (6×10^9/L) accompanied by 2 episodes of epistaxis. A platelet transfusion was administered and the platelet count normalized within 4 days of discontinuing HU. HU was restarted and the patient experienced no other thrombocytopenic episodes.

Eleven patients (13%) experienced 17 episodes of ALT elevation (Table 2). The mean ± SD for these abnormal ALT values was 166 ± 97 IU/L with a median of 125 IU/L and range from 60 to 393 IU/L. Sixteen episodes of ALT elevation resolved within 2 weeks and the other within 3 weeks. One patient with ALT elevations also had gallstones. In no instance was it possible to attribute an ALT elevation solely to HU treatment, analgesic usage, or clinical events. No patient experienced renal toxicity.

Table 2. Laboratory Toxicities During HU Therapy

Toxicities	No. of Occurrences	No. Patients With This Event	Percent of Visits With Toxic Value
Neutropenia	205	56	5.2
Reticulocytopenia	62	35	1.6
Anemia	43	27	1.1
Hepatic (ALT elevation)	17	11	0.4
Thrombocytopenia	13	7	0.3
Renal (creatinine elevation)	0	—	—

Table 3. Adverse Clinical Events Reported During HU Therapy

Type of Adverse Event	No. of Events	No. of Patients
Sickle cell-related events		
Vasoocclusive pain	76	34
ACS	10	8
Gallstones	1	1
Priapism	1	1
Splenic sequestration	1	1
Transient ischemic attack	1	1
Other clinical events		
Pain*	50	29
Nausea/vomiting	25	17
Infection	23	20
Headache	13	12
Diarrhea	7	6
Skin rash	7	5
Bleeding	2	1
Growth failure	0	0

*Pain reported by the patient that was not felt to be related to complications of sickle cell anemia by the patient and staff at participating center.

Table 5. Comparison of the Phase I/II Hematologic Values of Children and Adults With Sickle Cell Anemia Who Were Receiving Treatment at the End of Their Respective Studies

Variable	HUG-KIDS (mean Δ) n = 66*	Adult Phase I/II Trial (mean Δ) n = 32†
Hb (g/dL)	1.2	1.2
MCV (fL)	14	23
ARC (×10⁹/L)	−146	−158
WBC (×10⁹/L)	−4.2	−5.0
ANC (×10⁹/L)	−2.2	−2.8
Platelets (×10⁹/L)	−108	−83
Total bilirubin (mg/dL)	−1.0	−2.0
Hb F (%)	9.6	11.2
F cells (%)	31	45

*62 of the 66 pediatric patients had achieved MTD.
†26 of the 32 adult patients had achieved MTD.

Clinical adverse events. Table 3 summarizes clinical events reported during the study. There were 90 sickle cell–related events and 127 other events that were not ascribed to sickle cell anemia. The common sickle cell–related complications included PEs (76 events) and ACS (10 events). Thirty-four (40%) patients experienced PEs and 8 (10%) had an ACS. Priapism was reported once among the 44 males.

The clinical events that were not believed to be sickle cell–related included pain, headache, bleeding, gastrointestinal disturbances, unexplained fever, and minor infections. Fifty episodes of pain were not believed to be related to complications of sickle cell anemia, in the opinion of the patient and staffs at the participating centers (Table 3). The abdomen was the most common site of pain, although pain was also noted in the trunk, joints, and extremities. There were no clear precipitating factors for these painful events, although on many occasions the pain occurred along with vasoocclusive pain. No patient experienced alopecia. Five patients had skin rashes, but no rash was attributed to HU. There were 32 occurrences of mild gastrointestinal complaints, including 25 episodes of nausea and 7 of diarrhea. In 2 instances, nausea was ascribed to HU. Both patients had less nausea when HU was taken at bedtime. No patient had gastrointestinal complaints severe enough to warrant withholding HU treatment.

As illustrated in Table 4, HU did not have an adverse effect on growth. At each 6-month interval, then mean weight and height increased significantly ($P < .0001$). Four girls, ages 11.1 to 14.1 years, reached menarche while receiving HU. No patient became pregnant.

Comparison of HU in adults and children. The mean changes from baseline of hematologic values, total bilirubin concentration, and Hb F parameters in our pediatric population are compared with the patients in the adult Phase I/II HU trial in Table 5.[12] It was only possible to compare data on those patients who were receiving HU when their respective studies were closed. The laboratory changes for both patient populations are similar in all categories. The mean increase in the hemoglobin concentration was identical. Both groups had increases in the MCV, Hb F, and F cells. The WBC count, platelet count, ARC, ANC, and total bilirubin declined in both populations.

DISCUSSION

The HUG-KIDS trial addressed each of its aims. We showed that HU significantly increases the hemoglobin concentration, MCV, Hb F, and F-cell percentage above pretreatment values. In addition, we showed that pediatric and adult patients had similar hematologic toxicities. Finally, no adverse effect on growth was observed during the treatment period.

HU therapy produces a highly significant increase in the total hemoglobin concentration, MCV and MCH (Table 1). Each of these hematologic effects of HU in children is similar to those in adults (Table 5). The decrease in LDH, total bilirubin, and ARC suggest that the increase in hemoglobin concentration is associated with a decreased rate of hemolysis. HU causes an increase in the amount of Hb F and the percentage of red blood cells that contain Hb F. Increases in the intracellular Hb F concentration

Table 4. Growth Parameters (mean ± standard deviation) During HU Therapy

		6 mo		12 mo		18 mo		24 mo	
Variable	Entry Mean Value	Mean Value	Mean Change From Baseline	Mean Value	Mean Change From Baseline	Mean Value	Mean Change From Baseline	Mean Value	Mean Change From Baseline
No. of patients	84	78	78	76	76	71	71	35	35
Weight (kg)	32.3 ± 15.8	35.1 ± 17.1	2.4 ± 2.0	37.4 ± 18.0	4.5 ± 3.1	38.1 ± 18.2	5.9 ± 3.6	40.4 ± 19.9	8.4 ± 4.9
Height (cm)	134.7 ± 16.9*	137.8 ± 16.7*	2.7 ± 1.7*	139.6 ± 16.4*	4.8 ± 2.6†	140.8 ± 16.0	7.1 ± 2.9*	144.1 ± 17.3	10.1 ± 3.5

The mean values for both height and weight were significantly different from their respective previous values for each 6-month interval ($P < .0001$).

*Subtract 1 from N at top of column.
†Subtract 2 from N at top of column.

would be expected to reduce hemolysis by inhibiting Hb S polymer formation.

Most hematologic effects of HU on red blood cells were manifested within 6 months of initiating treatment (Table 1), well before the MTD was typically attained. In addition, there did not appear to be a substantial enhancement or diminution of these effects after 12 or 24 months of HU therapy. These observations have important implications. First, they suggest that frequent adjustments of the HU dose to reach the threshold of myelotoxicity may not be necessary. Second, once the patient is observed to respond to HU, there does not appear to be any evidence of "marrow exhaustion" during the treatment period of 1 year at MTD. Finally, a daily oral dose of 25 to 30 mg/kg generally is well tolerated by most children.

Patients continued to gain weight and height while taking HU for at least 1 year at MTD (Table 4). Further trials with HU being administered for longer periods of time, however, are needed to define with a greater degree of certainty the effects of HU on growth.

One to 2 years of HU therapy appears to be relatively safe in children with sickle cell anemia who are monitored closely by experienced pediatric hematologists. The most commonly observed toxicities were those related to its myelosuppressive effects (Table 2). These toxicities were transient and resolved quickly once HU was discontinued. Effects of HU on kidney and liver function were negligible during this study. Except for 1 episode of severe thrombocytopenia, there were no unusual or life-threatening toxicities observed during this study with over 100 patient-years of close monitoring. There remains the potential, however, that administration of HU for longer periods of time may be associated with major side effects that were not seen in this relatively short-term study.

Acute complications continued to occur in HUG-KIDS participants as they do in adult patients taking HU. Although we would expect that HU would reduce the frequency of acute complications and possibly prevent, or delay, organ damage in pediatric patients, the demonstration of such efficacy warrants a rigorous Phase III clinical trial. This trial also should attempt to define the complications related to long-term administration of HU.

ACKNOWLEDGMENT

APPENDIX

Children's Hospital, Boston: Susan Kurth, NP; Children's Hospital of Philadelphia: Lorien Moore, RN, Leslie Parkin, and Sonya Whitehead, RN; Duke University Medical Center: William H. Schultz, PA-C, MHS and Amy Walker, CCRA; East Carolina University: Diana Gordon, RNC and Cynthia Brown, CCRA; Oakland Children's Hospital: Ekua Hackney Stephens, MS, PNP; St Jude Children's Research Hospital: Kristy Cupples, RN and Lynn Wynn, RN; Memorial Hospital, University of North Carolina at Chapel Hill: Susan Jones, RN; Statistical Center, University of North Carolina, Department of Biostatistics and the Frank Porter Graham Child Development Center: Lisa Brooks, BA, Katherine Gover, MS, Elizabeth Gunn, BS, Mary Helms, BS, and Marsha McMurray, BS, MA.

REFERENCES

1. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, McMahon RP, Bonds DR: Effect of hydroxyurea on frequency of painful crisis in sickle cell anemia. N Engl J Med 332:1317, 1995

2. Scott JP, Hillery CA, Brown ER, Misiewicz V, Labotka RJ: Hydroxyurea therapy in children severely affected with sickle cell disease. J Pediatr 128:820, 1996

3. de Montalembert M, Belloy M, Bernaudin F, Gourard F, Capdeville R, Mardini R, Philippe N, Jais JP, Bardakdjian J, Ducrocq R, Maier-Redelsperger M, Elion J, Labie D, Girot R: Three year follow-up of hydroxyurea treatment in children with sickle cell disease. The French Study Group on Sickle Cell Disease. Am J Pediatr Hematol Oncol 19:313, 1997

4. Jayabose S, Tugal O, Sandoval C, Patel P, Puder D, Lin D, Visintainer P: Clinical and hematologic effects of hydroxyurea in children with sickle cell anemia. J Pediatr 129:559, 1996

5. Ferster A, Vermylen C, Cornu G, Buyse M, Corazza F, Devalck C, Fondu P, Toppet M, Sariban E: Hydroxyurea for the treatment of severe sickle cell anemia: A pediatric clinical trial. Blood 88:1960, 1996

6. Olivieri NF, Vichinsky EP: Hydroxyurea in children with sickle cell disease: Impact on splenic function and compliance with therapy. Am J Pediatr Hematol Oncol 20:26, 1998

7. Weinfeld A, Swolin B, Westin J: Acute leukaemia after hydroxyurea therapy in polycythaemia vera and allied disorders: Prospective study of efficacy and leukaemogenicity with therapeutic implications. Eur J Haematol 52:134, 1994

8. Wiger R, Hongslo JK, Evenson DP, De Angelis P, Schwarze PE, Holme JA: Effects of acetaminophen and hydroxyurea on spermatogenesis and sperm chromatin structure in laboratory mice. Reprod Toxicol 9:21, 1995

9. Kim HC, Adachi K, Schwartz E: Separation of hemoglobins, in Beutler E, Lichtman MA, Coller BS, Kipps TJ (eds): Williams' Hematology (ed 5). New York, NY, McGraw Hill, 1994, p L37

10. Horiuchi K, Fynn-Thompson F, Ohene-Frempong K: Quantitative analysis of the degree of irreversible deformation of F cells and non-F cells and its relationship to cell density in sickle cell disease. Exp Hematol 22:1058, 1994

11. Horiuchi K, Osterhout ML, Bekoe N, Kamma H, Bekoe NA, Hirokawa KJ: Estimation of fetal hemoglobin levels in individual red cells by fluorescence image cytometry. Cytometry 20:261, 1995

12. Charache S, Dover GJ, Moore RD, Eckert S, Ballas SK, Koshy M, Milner PF, Orringer EP, Phillips G Jr, Platt OS, Thomas GH: Hydroxyurea: Effects on hemoglobin F production in patients with sickle cell disease. Blood 79:2555, 1992