Hydroxyurea: Effects on Hemoglobin F Production in Patients With Sickle Cell Anemia


Patients with sickle cell anemia were treated with daily doses of hydroxyurea, to assess pharmacokinetics, toxicity, and increase in fetal hemoglobin (HbF) production in response to the drug. Plasma hydroxyurea clearances were not a useful guide to maximum tolerated doses of the drug. The mean daily single oral dose that could be maintained for at least 16 weeks was 21 mg/kg (range, 10 to 35 mg/kg). Among 32 patients, last HbF levels were 1.9% to 26.3% (mean, 14.9%) with increases in HbF over initial values of 1.4% to 20.2% (mean, 11.2%). The most significant predictors of last HbF were last plasma hydroxyurea level, initial white blood count and initial HbF concentration. Last HbF was not related to β globin haplotype or a globin gene number. No serious toxicity was encountered. Clinically significant bone marrow depression was avoided, and chromosome abnormalities after 2 years of treatment were no greater than those observed before treatment. The period of observation has been too short to evaluate the risk of carcinogenesis. Patient’s red cells developed striking macrocytosis. Median red cell Hb concentrations did not change. Hb concentrations increased, on average 1.2 g/dL, but serum erythropoietin levels increased. Patients’ body weights increased, and some returned to work or school, but no conclusions regarding therapeutic efficacy could be drawn from this uncontrolled open-label study.

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MATERIALS AND METHODS

Informed Consent and Protection of Patients

Written informed consent was obtained from all patients, using forms and procedures approved by local Institutional Review Boards. All consent forms included statements that the study drug might be carcinogenic, although the risk was considered low, and that other unforeseen untoward effects could occur. The study was overseen by an independent Policy Board, as well as by NHLBI staff under the terms of a Cooperative Agreement.

Organization and Eligibility Requirements

Seven institutions participated and a coordinating center and core laboratory were set up at Johns Hopkins Hospital. After approval of a common protocol by Institutional Review Boards at each site, coordinators were trained in necessary procedures, and recruitment began. Only adult patients (age 18 years or older) with

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sickle cell anemia were included; electrophoretic patterns showed only Hb S, F, and A2. They must have had at least two crises in the year before entering the study (including emergency room visits) and could not be participants in a chronic transfusion program. Patients with known S/beta-thalassemia were excluded to maintain homogeneity of the study group.

Other exclusion criteria were aspartate amino transferase (AST) greater than 100 IU/L; albumin less than 3 g/dL; creatinine greater than 2 mg/dL; narcotic addiction (but not medically supervised use of narcotics); pregnancy or unwillingness to use contraceptives; use of prophylactic drugs (because of relating xanthines to the pharmacology of HU),10 androgens, estrogen, or progestins (other than in birth control pills); and human immunodeficiency virus (HIV) infection. Patients were told that noncompliance with instructions would lead to their being dropped from the study.

Drug Dosing

HU was supplied by SquibbMark (Evansville, IN), the manufacturer, as a powder, and encapsulated into differently colored 100 and 500 mg capsules by The Johns Hopkins Pharmacy. Capsules were assayed by the method of Fabricus and Rajewsky.14

Drug dosage was increased by 20 mg/kg for AUC6 less than 1,000; 50 mg/kg for AUC6 greater than 1,000. Aliquots of frozen urine and plasma were shipped to Baltimore for assay by the method of Fabricus and Rajewsky.14

Two weeks after completion of the second clearance, treatment was begun. A single daily dose 1 hour before eating was used.

Drug dosage was increased by 5 mg/kg every 8 weeks, unless toxicity was present. At each dose level blood counts were performed on weeks 1, 2, 3, 5, and 7. Toxicity was initially defined as less than 2 x 10^9 neutrophils/L, less than 100 x 10^9 platelets/L, less than 125 x 10^9 reticulocytes/L, a 20% decrease in Hb concentration, or an absolute value of less than 0.5 g/dL. 50% increase in serum creatinine or an absolute increase of greater than 0.4 mg/dL, a 100% increase in alanine aminotransferase (ALT) G1 disturbance, or rash or hair loss not attributable to other causes. If toxicity was encountered, the drug was stopped until toxicity cleared, and then was resumed at the same dose. If toxicity recurred, the dose was decreased by 5 mg/kg, and resumed after toxicity cleared again.

Treatment began in February 1988. In September 1988, when 27 patients were under study, only hematologic toxicity had been encountered, but the definition of toxicity was considered overly cautious. Critical levels were reduced to neutrophils less than 2 x 10^9/L, platelets less than 20 x 10^9/L, and reticulocytes less than 80 x 10^9/L if the Hb concentration was less than 9 g/dL. Further, if toxicity recurred after a dose was resumed, treatment was stopped until blood counts had returned to the pretreatment level, rather than the “nontoxic” one. Drug administration was then resumed at a dose 2.5 mg/kg below the toxic dose. An attempt was made to find and continue a maximum tolerated dose (MTD) for at least 16 weeks. In 12 patients who completed 16 weeks at the MTD, a further 16 weeks of treatment was attempted, using the same total amount per day, but dividing it into two doses taken at intervals of about 12 hours.

Laboratory Studies

At each visit, a sample was obtained for automated blood count (including mean corpuscular volume [MCV]), manual differential white blood cell (WBC) count, and reticulocyte count. Results from the former tests were obtained by accredited hospital laboratories and mailed to Baltimore. Blood for the reticulocyte count was shipped to Baltimore by air express and assayed by flow cytometry, using a method which permits testing as long as 1 week after blood sampling. The sample was used for assay of HbF,13 F cells,16 and F reticulocytes.16 The HbF assay used is known to underestimate high HbF levels to a small degree. The amount of HbF per F cell (F/F cell) was calculated. At 3-month intervals, phthalate ester density centrifugation was performed to assay dense cells (mean corpuscular Hb concentration [MCHC] > 36 g/dL) and median RBC Hb concentration. One center used a Technicon H-1 blood cell counter (Technicon, Tarrytown, NY), which directly analyzes the Hb concentration within individual RBCs.18 Price-Jones curves were constructed using a Hematrat 360 (Diagnostics Division, Beckman Instruments, Inc, Brea, CA).

During week 2 at each dose level patients spent 8 hours in the participating clinic. HU was swallowed under observation, blood was collected 2 hours later, and urine was collected for 8 hours. Frozen plasma (“2-hour plasma”) and urine were sent to Baltimore for assay, to assess pharmacokinetics during dose escalation. Random plasma samples (“random plasma”) were obtained on weeks 3 and 5 at each dose level as a measure of compliance (without a uniform time period between drug ingestion and blood sampling), and assayed for HU.

At the beginning and end of the study, heparinized blood was sent to Baltimore for karyotype analyses.19 Beta chain cluster haplotypes and a gene numbers were assessed by established methods.20 Serum samples were obtained for erythropoietin (Epo) assay before treatment and at conclusion of the study. These were stored at −70°C and assayed in batches by T. Buckholz of the Drug Metabolism division of the R.W. Johnson Pharmaceutical Institute (Raritan, NJ) using a radioimmunoassay,21 through the courtesy of Dr I. Smith and J. Boccagno. Serum ferritin, folate, and B12 were assayed at the beginning of the study at each clinic, using standard radioimmunoassays.

Painful Crises

An outpatient crisis was defined as pain lasting more than 2 hours requiring oral narcotics for relief. In-patient crises were admissions decided on by physicians other than the investigators, for any vaso-occlusive event, and excluding gout, gallbladder disease, or infection. No attempt was made to confirm reports made by patients, to assure that all episodes (including those treated at nonparticipating hospitals) were reported, or to be certain that each clinic adhered to the foregoing definitions. Nine patients completed the short form of the Medical Outcomes Study,22 an instrument designed to assess the results of medical care, including functioning in daily living and perceptions of patient’s general health and well being.

Transfusion, Surgery, and Pregnancy

All investigators agreed that blood transfusions would be administered only for severe anemia, but in several instances patients did receive blood at nonparticipating hospitals. In such instances, treatment was halted for 3 months, and then resumed. Treatment was continued during crises. One patient underwent surgery during the study. HU administration was interrupted only during the period when oral intake was stopped. No problems with wound healing or other aspects of the postoperative course were encoun-
ttered. Women were asked for the date of their last menstrual period on each visit, and tested for pregnancy if amenorrhea or any menstrual irregularity developed. Men were asked about contraception, and any potential pregnancy in sexual partners, on a regular basis. Counseling was given if conception occurred.

**Statistical Methods**

In Baltimore, data from the clinics were edited and corrected for omissions and transcription errors, and then analyzed using SAS (SAS Institute, Inc, Cary, NC).

The paired t-test was used to compare initial with last measures for individual patients. The Pearson correlation coefficient was used to examine bivariate associations between continuously defined variables. Stepwise multiple linear regression was used to examine the simultaneous association of several variables with therapeutic outcome. In that technique, all variables of interest are examined simultaneously. The most highly correlated variable is entered into a regression equation, and then the process is repeated, choosing the next most highly correlated variable. Reiterations continue until no remaining variable meets a preset criterion, in this case $P \geq .15$. The method differs from classical linear regression in that poorly related, or nonrelated, variables are omitted from the final equation derived by the stepwise procedure. All $P$ values were two-sided. Because of the small number of patient studied, and the large number of analyses performed, $P = .02$ to .01 was considered "probably significant," and $P < .01$ was considered "significant."

Many data are expressed as initial values (for instance, the average of three pretreatment measurements, HbF$_{i0}$, averaged values obtained while the patient was receiving the MTD (Last F, for instance), and the difference between last and initial values ($\Delta F$). Similar designations are used for other variables that were treated in the same fashion (Hb$_{i0}$, MCV$_{i0}$, Last MCHC, Last Epo, $\Delta F$/F Cell, etc).

**RESULTS**

**Patients Enrolled**

Forty-nine black patients (27 men and 22 women) were enrolled, and 32 (18 men and 14 women) were under treatment at the end of the study. Their mean age was 27.6 years (SD, 6.3). Of the 32, 26 were treated with an MTD for 2 to 38 weeks. Eighteen patients were treated for 24 months or longer, and 11 completed 16 weeks on an MTD and were treated on a divided dose (see above). Patients were treated for 0 to 771 days (mean, 277), one patient having withdrawn from the study before actually beginning treatment.

Reasons for withdrawing from the study were: eight patients did not return to clinic for at least 2 months; four moved to another city or lost means of transportation; three were repetitively transfused at peripheral hospitals; one developed narcotic dependency; one became HIV positive; and one died. Patients who left the study did so after 1 to 83 weeks of treatment (mean, 29.7). Their previous crisis history, HU doses at the time of departure, and age were not significantly different from those of patients who remained in the study. When compared with the two patients who entered the study immediately before and after they did, Hb F levels at the time of drop-out were lower in the dropouts (mean HbF, 7.9% vs 11.1%; $P = .02$), but the number of crises since entering the study did not differ between those who left and those who remained.23

The single death in a study patient occurred after 9 weeks of treatment, his dose having just been increased to 20 mg/kg 1 week before death. The patient had a seizure followed by irreversible ventricular fibrillation, during an admission for back and leg pain (hematocrit [Hct] 18%). There was neither laboratory nor clinical evidence of HU toxicity. At autopsy, there was recent ischemic damage in the left frontal cortex of the brain, but no evidence of cerebral vascular disease, hemorrhage, or thrombosis.

**Initial Laboratory Studies**

Initial laboratory studies of the patients who completed the study are summarized in Table 1. Unless specified otherwise, all subsequent data refer to these 32 patients. Initial MCV was positively correlated with initial reticulocyte count ($r = .70, P = .0001$) and with $\alpha$ globin gene number ($r = .62, P = .0002$). The mean initial HbF level was 3.8% (range, 0.2% to 12%). It was correlated with percent F cells and percent F reticulocytes ($P < .0001$), but not with HbF/F cell ($P = .07$). Initial HbF was not correlated with any other baseline measurement, including serum ferritin, folate, vitamin B12, urea nitrogen, Epo, $\beta$ globin haplotype, and $\alpha$ globin gene number. $\beta$ globin gene haplotypes are given in Table 2. Thirty-two percent of the patients had three $\alpha$ globin genes, and 68% had four. HbF levels did not differ between men and women (3.2% vs 4.6%, $P = .11$).

**HU Clearance Studies**

In most patients, duplicate plasma HU clearance studies yielded similar results, but urinary excretion of HU was more variable. Plasma levels peaked at 3 to 4 hours after drug ingestion. Mean plasma level at 2 hours was 19 $\mu$g/mL. Mean cumulative urinary HU excretion was 62% of the administered dose at 8 hours.

The mean creatinine clearance was 165 mL/min (SD, 95) and the mean AUC6 was 1,216 (SD, 397) $\mu$g mL/h. AUC6 was correlated with patients' age ($r = .47, P = .007$), but

| Table 1. Comparison of Pretreatment Measurements With Measurements at MTD |
|---------------------------------|----------------|----------------|
| Pretreatment Value | Value at MTD | $P^*$ |
| HbF (%) | 4 ± 2 | 15 ± 6 | .0001 |
| F reticulocytes (%) | 8 ± 5 | 23 ± 10 | .0001 |
| F cells (%) | 28 ± 14 | 73 ± 17 | .0001 |
| F/F cell (pg) | 5 ± 2 | 8 ± 2 | .0001 |
| Enrichment ratio | 4.1 ± 2.6 | 2.6 ± 0.8 | .0001 |
| Hb (g/dL) | 8.5 ± 1.4 | 9.7 ± 1.8 | .0001 |
| Reticulocytes ($\times 10^9$/L) | 401 ± 157 | 243 ± 73 | .0001 |
| MCV (fL) | 94 ± 6 | 117 ± 15 | .0001 |
| Median CHC (g/dL) | 34 ± 3 | 34 ± 2 | .39 |
| Epo (U/L) | 202 ± 190 | 471 ± 873 | .03 |
| WBC (cells $\times 10^9$/L) | 13.4 ± 3.2 | 8.4 ± 1.4 | .0001 |
| Neutrophil count (cells $\times 10^9$/L) | 7.4 ± 2.7 | 4.6 ± 1.1 | .0001 |
| Platelets ($\times 10^9$/L) | 447 ± 136 | 364 ± 73 | .0003 |
| Total bilirubin (mg/dL) | 3.9 ± 3.4 | 1.9 ± 1.2 | .0001 |
| ALT (IU/L) | 36 ± 33 | 37 ± 29 | .78 |

Values are mean ± standard deviation.

*P by paired t-test.
was not correlated with baseline serum creatinine or creatinine clearance. During the course of the study, the plasma HU level 2 hours after drug ingestion was correlated linearly with drug dose ($r = .48, P < .0001$) (Fig 1), with a mean value for 25 mg/kg doses virtually identical to the mean 2-hour blood level measured during AUC6 measurements.

The dose at first toxicity (mean, 22.1 mg/kg; range, 15 to 35) was inversely correlated with AUC6 ($r = -.42, P = .016$) but was not correlated with patients' age, baseline serum creatinine, or creatinine clearance ($P > .20$). The MTD (mean, 21.3 mg/kg; range, 7.5 to 40) was not correlated with AUC6 ($P = .31$). Men tolerated higher doses than women (mean MTD, 24.2 v 17.5 mg/kg; $P = .01$).

### Changes in HbF synthesis
Not infrequently (22 definite examples/46 episodes), increases in HbF level were observed at the times toxicity was noted. These were considered due to decreased erythropoiesis and preferential survival of previously synthesized F cells, and HbF levels obtained at times of toxicity are not considered with the following data.

HbF levels increased, to some degree, in all patients (Fig 2), as did F cells, F reticulocytes, and F/F cell ($P = .0001$). Last F levels ranged from 1.9% to 26.3% (Table 3), with $\Delta F$ of 1.4% to 20.2% (mean, 11.2; SD, 5.2). In 16 patients, HbF began to increase by the second clinic visit 4 weeks after treatment was started. In 15 others, there was a lag of between 4 and 12 weeks between treatment and response, and in one a response was virtually absent. The starting doses did not differ between those patients who did and did not respond promptly, and the average dose in the two groups was the same (15.6 v 15.8 mg/kg). There were differences between patients in the persistence of response during treatment. In several instances, noncompliance was suspected, and responsiveness to treatment appeared to increase when the topic was discussed with some of the patients involved.

During dose escalation, the average HbF level was correlated with dose (Fig 3), but Last F and $\Delta F$ were not

### Table 3. Prediction of HbF Response

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial $r^2$</th>
<th>Model $r^2$</th>
<th>Probability</th>
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</thead>
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<td>$\Delta F$</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
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<td>.39</td>
<td>.0001</td>
</tr>
<tr>
<td>Last plasma HU</td>
<td>.11</td>
<td>.50</td>
<td>.02</td>
</tr>
</tbody>
</table>

Variables: zero random plasma percent; last plasma HU, HbF0, F cell, F retic, HbF0, weight, age, sex, AUC6, Cr, Sel, Abs Retic0, WBC0, ALT0, PLT0, Epo0, last Epo,дол, $\Delta F$.

Abbreviations: HbF0, initial HbF; ALT0, initial ALT; Zero random plasma, fraction of random samples containing no HU; Last plasma HU, last plasma HU level.
HYDROXYUREA: EFFECTS ON HbF PRODUCTION

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Fig 3. HbF levels for all patients at all doses. Each patient is represented several times. Average HbF level was correlated with dose \((r = .32, P = .0001)\) and plasma HU level \((r = .39, P = .0001)\).

correlated with final dose (Fig 4). Last F and \(\Delta F\) were significantly greater for patients receiving \(\geq 17.5\ mg/kg\) than for those receiving less than that dose \((P = .0009)\), and Fig 4 suggests a plateau in HbF response at higher doses. In 7 of 32 patients, HbF levels remained the same, or decreased, as dose was increased; four of the seven reached their maximum HbF levels at doses greater than 17.5 mg/kg. Noncompliance at higher doses was suspected in one patient (102), because a number of random samples of his plasma contained no HU.

F reticulocyte and F cell counts increased as dose of HU increased (Fig 5), while the “enrichment ratio” (F cells/F retics) decreased (Table 1). The change in enrichment ratio was correlated strongly with both Last F \((r = .64, P = .0001)\) and \(\Delta F\) \((r = .57, P = .0006)\). At the MTD, F cells showed a mean increase of 45% (SD, 20%). The increase in F cells during treatment (160% of baseline) was greater than the increase in HbF/F cell (78% of baseline).

Measuring individual bivariate associations, \(\Delta MCV\) during treatment was strongly correlated with \(\Delta F\) (Fig 6) \((r = .68, P = .0001)\). Last F and \(\Delta F\) were strongly correlated with last “2 hour” plasma HU levels, and \(\Delta F\) was correlated with last dose (if patient 102, for whom 40 mg/kg was prescribed, was excluded from analyses; see Compliance below).

Because some patients showed better responses than others to HU, pretreatment measurements were analyzed as potential predictors of Last F. Last F, \(\Delta F\), and Last F/F cell were correlated with initial WBC \((P < .01)\). Detailed tables of the results of these analyses are available from S.C. on request. Initial WBC was not correlated with MTD \((P = .12)\), but if patients 102 and 402 were excluded (see Compliance), there was a strong correlation between the two variables \((r = .56, P = .0013)\).

Neither \(\beta\) globin gene haplotype, nor presence or absence of an \(XmnI\) site 158 bases upstream from the G \(\gamma\) gene locus were associated with either Last F or \(\Delta F\). \(\alpha\) Globin gene number was not associated with increase in F cells or F reticulocytes but patients with four genes showed larger increases in HbF/F cell \((P = .018)\). Increases in HbF production were not correlated with absolute reticulocyte count, platelet count, initial serum folate, vitamin B12, or ferritin levels.

The significance of the various determinants of Last F was addressed by stepwise multiple linear regression analyses (Table 3). Examining a wide variety of independent variables, the most important were last 2-hour plasma HU level, and initial HbF and WBC. The total variance \((r^2)\) explained by the model was 59%. Absolute neutrophil count and absolute mononuclear cell count were not associated. When \(\Delta F\) was the dependent variable, findings were similar except for omission of initial HbF level.
The five patients who showed the smallest increases in HbF (Fig 2, “poor responders” in Table 4) were reassessed in December 1991, 14 months after completion of the study. Patient 102 said he continues to take 40 mg/kg, but his HbF had decreased and his MCV was 92 to 101 fL. Patient 411 had his dose increased; his HbF increased somewhat. Patient 503 continued treatment for 9 months. Her crises ceased to occur but she then stopped treatment because she wanted to become pregnant; her HbF decreased, and crises recurred, including a life-threatening episode of chest syndrome. Patients 510 and 511 continued HU for 9 months, but then stopped because they could not afford to pay for it themselves. During that period, patient 510’s total Hb increased from 5 to 8 g/dL, and she developed aseptic necrosis of the femoral head. The one patient with a lower MCV than a “nonresponder.” Bearing in mind that the “last F” values of Table 4 were not measured in the central laboratory, all patients except 102 showed definite increases in HbF production during treatment, and higher HbF values in December 1991 than they had had at the end of the study.

Changes in Patients’ RBCs During Treatment

RBC volume increased during treatment, with Last MCV (117 fL; range, 91 to 146) and Δ MCV (23 fL; range, –2 to 44) being higher in those with four α globin genes (P = .002) and correlated strongly with dose (Fig 7). Δ MCV was correlated with Δ MCH (r = .49, P = .007). MCV increased much more than did RBC diameter on blood smears (Fig 8). The values used for MCV were derived from electronic cell counters, but such MCVs were very strongly correlated with MCVs calculated from MicroHct/RBC (r = .85, P = .001). MicroHcts and calculated packed cell volumes were also closely correlated (r = .89, P = .0001).

Reticulocyte counts decreased, but remained higher than normal (Table 1). Median corpuscular Hb concentrations (CHC) did not change significantly but the distribution of CHCs in individual patients narrowed. That narrowing probably was related to virtual disappearance of dense cells during treatment (from a mean of 3% ± 5% to 0.8% ± 1.4%), and a decrease in numbers of relatively hypodense reticulocytes. There was no correlation between Δ F and a decrease in dense cells (P = .77) or change in median CHC (P = .65). Irreversibly sickled cells disappeared from blood films, paralleling the loss of dense cells (Fig 8). RBC diameters, before and at the end of treatment, were compared by means of Price-Jones curves. The mean diameter of five patients’ cells before treatment was 6.78 μm, and at the end 7.24 μm. The mean difference between samples was 0.46 μm, a 6.8% increase, compared with the 24.5% increase in cell volume.

Most patients’ total Hb concentrations increased (P = .0001) as HU dose increased, the mean increase being 1.2 g/dL, but serum Epo levels were higher at the end of treatment (Table 1). Unlike the results of comparisons with initial Epo level, Last Epo was correlated with Last F (r = .41, P = .02), Last F/F cell (r = .52, P = .003), and Last plasma HU (r = .47, P = .006). Correlations with Δ

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<th>FO</th>
<th>Max F</th>
<th>MTD F</th>
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Abbreviations: FO, Initial HbF; Max F, highest HbF during treatment; MTD F, Hb F while receiving MTD; ΔF, 0 – MTD F; MCV, initial MCV; Max MCV, maximum MCV; MTD MCV, MCV while receiving MTD; Last Dose, last known dose; Last ΔF, initial – Last HbF.
Epo were very similar to correlations with Last Epo. Mean serum total and indirect bilirubin levels decreased (2.3 and 1.3 mg/dL, respectively, \( P = .0001 \)), a change consistent with decreased hemolysis, but changes in those measurements and in serum AST and ALT levels were not correlated with changes in HbF synthesis.

**Dose Adjustment and Toxicity**

The only toxicity encountered was bone marrow depression. None of the clinics encountered an increase in infections in study patients, or any unusual infections, including viral infections (with the exception of the one patient who developed HIV infection and was dropped from the study). There was no suggestion that HU therapy was hazardous in patients with mild liver disease (ALT < 100) and that exclusion criterion was probably unduly cautious.

The mean time to occurrence of the first episode of toxicity was 8.0 weeks (range, 4 to 14) using the original definition of toxicity (\( N = 8 \) patients), and 20.7 weeks (range, 5 to 38) (\( N = 23 \)) using the final definition. All but two patients became toxic again when a previously toxic dose was repeated; those two patients were suspected of noncompliance (see below). The most common form of toxicity was neutropenia (73%), followed by reticulocytopenia (22%), both neutropenia and reticulocytopenia (3%), thrombocytopenia (1%), and a decrease in HB concentration (1%). One patient developed severe anemia (Hb 3.8 g/dL) and reticulocytopenia, but did not require hospitalization or transfusion and recovered promptly. The average duration of toxicity was 2.7 weeks for return to “nontoxic” blood counts, and 4 weeks for return to pretreatment counts, only 5% of patients having recovered by the next clinic visit (2 weeks after toxicity was noted).

At the MTD the means of patients’ blood counts were within the normal range (with the exception of HB concentration and Hct), although they were lower than initial counts (Table 1). The change in WBC was correlated with \( \Delta F, \Delta F/F \) cell, and \( \Delta F/F \) cell (\( r = -0.67, -0.55, -0.50; P = .0001, .001, .005 \)). Changes in platelet and reticulocyte counts did not show such a correlation. Because patients at their MTDs were treated to equal degrees of toxicity, it was anticipated that their absolute neutrophil, platelet, and reticulocyte counts would not differ significantly at their MTDs. That proved to be the case.

Twelve patients were studied while taking the drug in two divided doses. In nine, HbF levels decreased, in one it decreased slightly, and in two the HbF level did not change. When directly questioned, a number of patients admitted that they frequently forgot to take their second dose of the day.

Despite urgings to use contraception, two women became pregnant during the study. Both terminated their pregnancies. Three male patients fathered children during the study. One partner terminated the pregnancy, and one continued and had a normal child, which is now 2 years old. The third patient’s partner is currently pregnant; the patient states that conception occurred while he was not taking the drug because of toxicity, and the couple wish to continue the pregnancy. Because of concern over the clastogenic (chromosome-breaking) effect of HU, karyotypes were prepared from peripheral blood lymphocytes at the beginning and end of the study. The overall number of abnormalities was no greater at the end of the study than it had been at the beginning (Table 5), and the number of abnormalities seen was not related to the final dose of HU.

**Compliance**

The association between compliance and HbF response was examined by examining the correlation between absence of any HU in “random” plasma samples and HbF response. The proportion of samples with no HU was correlated strongly with Last \( F \) cell (\( r = .50, P < .01 \)) and \( \Delta F \) cell (\( r = .53, P < .01 \)), less significantly correlated with \( \Delta F \) and \( \Delta F/F \) cell (\( P = .02 \)), and not correlated with other measurements of the HbF response.

Two patients appeared able to tolerate doses as high as 37.5 to 40 mg/kg. However, one of them (102) had developed toxicity at a dose of 25 mg/kg, and the other (402) had developed it at 20 mg/kg, but neither ever again became toxic or showed a further increase in HbF. Both were suspected of taking their capsules irregularly because many of their random plasma samples contained no HU, but noncompliance was not proved in either of them.

**Clinical Responses**

**Weight gain and return to work.** All of the 14 women gained weight (mean, 5.0; range, 0.9 to 11.2 kg) as did 12 of the 18 men (mean, 4.3; range, −2.0 to 12.5 kg) (for all patients, \( P = .0001 \)). Δ Weight was not correlated with Last \( F \) or \( \Delta F \) (\( P = .23, .16 \)) or with initial weight or dose of HU. Eight of the patients returned to school or work. Nine subjects completed the Medical Outcomes Study; average

<table>
<thead>
<tr>
<th>Table 5. Karyotype Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormality</td>
</tr>
<tr>
<td>Chromatid gaps</td>
</tr>
<tr>
<td>Centromere gaps</td>
</tr>
<tr>
<td>Centromere breaks</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Total abnormal</td>
</tr>
</tbody>
</table>

Differences between initial and final chromatid breaks, chromosome gaps, chromosome breaks, translocations, deletions, inversions, and aneuploidy were not significant.
scores for those patients improved in 15 of 20 items when pretreatment and posttreatment responses were compared.

Painful Crises

As noted above, the patients who dropped out of the study did not have more crises at the time of drop-out than those who remained. Among the patients who completed the study, the mean number of emergency room visits in the 6 months before the study was 4.0 (range, 0 to 20), the mean number of hospitalizations was 2.1 (range, 0 to 6), and the mean number of days spent in the hospital was 19.2 (range, 0 to 94). During treatment, attack rates for reported painful crises during successive 6-month blocks decreased (Table 6). None of the differences in attack rates were significant ($P > .05$) when patients were compared with themselves (first 6 months v last, for instance). The number of unreported crises is unknown, and these data must be interpreted with caution. The decrease in crises was not correlated with either mean HbF levels during each 6-month block or changes in HbF levels.

DISCUSSION

Changes in HbF Synthesis

Last plasma HU level, initial HbF, and initial WBC count were the most important predictors of Last F in stepwise regression analyses. The first suggests that the more HU one can safely administer to a patient, the better his HbF response will be (if he takes his capsules); the data in Fig 4 are not entirely consistent with that interpretation. The second is consistent with the hypothesis that there may be common determinants of both baseline HbF and posttreatment levels. The last may reflect or predict bone marrow "reserve" (ie, resistance to myelosuppression) and is supported by the finding of a strong correlation between initial WBC and MTD. Viewed from a different perspective, patients whose initial WBC were high may have had increased production of or sensitivity to hematopoietic growth factors (interleukin-3 [IL-3] and granulocyte-macrophage colony-stimulating factor [GM-CSF]), both of which have been shown to increase HbF production in animals.24 A variety of interactions between HU and nearly ubiquitous chemicals might have affected the responses we observed. Both caffeine10 and aspirin20 could conceivably alter the effect of HU on γ chain synthesis, as could the amounts of iron and iron chelators included in patients' diets.26

Neither α globin gene number nor β globin haplotype (including the Xmn I polymorphism 158 bases upstream from the $^\gamma$ gene)27 were related to response. As seen earlier, the proportional increase in F cells was greater than that in Hb F/F cell, thereby accounting for the decrease in enrichment ratio. The mechanism by which RBC precursors are diverted into the F cell line is unclear, except for the clear observation that increases in HbF were related to HU dose administered. Although an increase in HbF showed a correlation with a decrease in WBC, there was not correlation with a decrease in reticulocytes, making premature "recruitment" of RBC precursors28 an unlikely explanation.

Our results expand those obtained by Rodgers et al.29 Our patients were treated for a much longer period of time, in an open environment, but we encountered no unexpected toxicity and our patients' HbF levels increased to considerably higher levels than theirs, despite suspected problems with compliance. Rodgers et al encountered a lag period before HbF increased; we saw a lag in only about half of our patients, perhaps because of a difference in dosage regimens (their patients having received intermittent therapy, which we showed earlier30 to be less effective than daily treatment). Several of our patients exhibited minimal increases in HbF, as did two of those reported by Rodgers et al. Noncompliance was suspected, but not proved, in some of our nonresponders, but could be ruled out in Rodger's patients, who were in-patients at the time of study. On the other hand, Rodgers et al's "nonresponders" showed less change in WBC and MCV than did ours, suggesting that the doses they used were suboptimal. As with our patients, no predictors of a good response were identified in that study. The search for determinants of "F-cell responsiveness" must continue, but it is not clear whether nonresponsiveness reflects anything more than low plasma HU levels, due either to a prescribed dose that was too low or noncompliance.

Serum Epo levels increased during the study, despite an increase in Hb concentration. That change could well reflect an increase in oxygen affinity, due to increased HbF in RBCs and decreased polymerization of HbS,31,32 or might have been due to mild bone marrow depression produced by HU.33 Recent data have cast some doubt on whether exogenous Epo can add to the effect of HU,34 and potentially permit lower HU doses and less toxicity. The exact dosage regimens used use of other hematinics, and perhaps even the time of day that drugs are administered, may play some role in those responses.

The late follow-up data in Table 4 support the hypothesis that final HbF depends on initial HbF and the amount of drug prescribed and taken, but suggest that HbF levels may continue to increase over a long period of time. They also suggest that we may have been overcautious in our definition of MTD. They cast further doubt on the concept of "nonresponder," and suggest that all patients can respond, to a lesser or greater degree, although last HbF levels may not be in a range usually thought to be associated with clinical benefit.

### Table 6. Painful Crises (in- and out-patient crises combined) at 6-Month Intervals

<table>
<thead>
<tr>
<th>Interval</th>
<th>No. of Crises</th>
<th>N</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>32</td>
<td>2.1 ± 3.2</td>
<td>0-11</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td>32</td>
<td>1.3 ± 2.0</td>
<td>0-9</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>27</td>
<td>1.3 ± 2.5</td>
<td>0-10</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>18</td>
<td>0.8 ± 1.1</td>
<td>0-4</td>
</tr>
</tbody>
</table>

Probabilities of differences between patients in various intervals: 1 v 2, $P = .06$; 27 patients in interval 3 versus the same patients in interval 1, $P = .19$; 18 patients in interval 4 versus the same patients in interval 1, $P = .07$. 

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Changes in RBCs

Macrocytosis was a striking result of HU therapy and paralleled the increase in HbF synthesis, as observed by others.34 That the change in MCV was not simply a result of antimitabolite therapy is suggested by a parallel between MCV and HbF in patients who had not been exposed to chemotherapy.35 The increase in MCV was correlated with increases in MCH. Cell volumes increased more than did cell diameters, as noted by others,36,37 suggesting that cells became more spherical during treatment, perhaps because cell surface area could not continue to increase as cell volume was expanded by increasing Hb content. Median CHCs did not change, indicating that the increase in MCH was associated with proportionally increased cell water. The proportion of dense cells decreased markedly, possibly because of increased HbF production.38,39 Because not all RBCs contained HbF, and the distributions of MCV and MCHC were unimodal, it seems likely that HU therapy stimulated synthesis of HbS in the non-F cells.

We did not measure RBC survival in our patients, but others have shown that it is increased in SS patients receiving HU.12,40 The same probably occurred in our patients and may account for the decreased bilirubin and increased total Hb concentrations that were seen.

Dose Adjustment and Toxicity

Earlier studies suggested that HU clearance might be a useful guide to starting dose of HU, based on relatively short-term observation. It did not prove helpful in a long-term study, and in future trials we plan to start patients just below the average MTD, with an initial dose of 20 mg/kg. The data in Fig 4 could be interpreted as showing no advantage in giving patients doses ≥17.5 mg/kg. There is much variation in Last F values at each dosage level, and of the seven patients who showed a “plateau” in response as dose was raised, four of them reached that point at doses greater than 17.5, suggesting that it is reasonable to try to maintain patients at their MTDs, as was done here.

Some patients, during the later phases of the study, developed toxicity as long as 16 weeks after beginning a dose. Based on the experiences reported here, it would seem entirely safe to check patients’ counts at 2-week intervals, and perhaps longer after prolonged treatment, but it would seem unlikely that surveillance of blood counts could ever be stopped entirely in patients on chronic therapy. Because patients almost invariably developed toxicity again if a previously toxic dose was tried again, after marrow recovery, it would seem wise to lower a patient’s dose whenever toxicity is encountered.

Using our modified criteria for toxicity, use of HU was not associated with bleeding, or any more than common infections. One patient developed a short self-limited episode of severe anemia. There was no evidence to suggest that it was a virus-related aplastic crisis, and although reticulocytopenia was a relatively uncommon form of toxicity, reticulocyte counts must be included in the list of regular required blood tests. Flow cytometric counts, because of their increased precision over manual counts, are the method of choice.

Long-term hazards of therapy are uncertain. Chromosome damage, considered minor in earlier work,7 is probably not a serious problem. Teratogenesis and mutagenesis are unknown risks, and every effort should be made to prevent conception by either men or women participating in future trials. The risks are very poorly documented,6,41,42 if they exist at all, but cannot be ignored. There are no reliable data to implicate the drug in carcinogenesis. Useful data on the point will eventually come from studies of HU in patients with polycythemia vera, and from long-term follow-up of the patients reported here, because all have elected to continue HU therapy. Until reliable statements can be made, patients should be warned of the risk. Because of the unknown effect of chronic HU therapy on growth and development, as well as the possible risk of carcinogenesis, balancing of risks may be very difficult in adolescents (or younger children) who have proved refractory to all other therapy; careful discussion between parent, child, and physician would seem the only potential resolution to such dilemmas.

Compliance Assessment

In planning the study, plasma HU levels were chosen to assess compliance. They were the most important predictors of Last F and Δ F; the five “poor” reactors in Fig 2 had significantly more zero plasma levels than did the remainder of the study group. Two divided doses were less effective than a single daily dose, as others have found,43 perhaps because patients forgot their second dose of the day. An alternate explanation would relate lower HbF values to the lower peak plasma HU levels that would have resulted from divided doses.

Patient compliance with the therapeutic regimen is an important variable. Compliance appears particularly important in evaluating the usefulness of a drug that acts as slowly as HU and that must be continued indefinitely if therapeutic effects are to persist. In any future evaluation of clinical efficacy of HU, it would seem essential to incorporate some measure of compliance into study design.

Clinical Responses

Data from the CSSCD imply that any increase in HbF synthesis might be beneficial,9 but clinical experience suggests that some of the responses we observed were too small to be meaningful. We could not select a “therapeutic” HbF level before this study began, and we cannot do so now. The preliminary nature of our investigation, and the costs of conducting a careful therapeutic trial, precluded rigorous definition of crises, use of blinded placebos, and efforts to ascertain all painful events. The difference between attack rates for individual patients during the time intervals shown in Table 6 are not significant.

One could argue that the 18 patients who completed 2 years of therapy were self-selected (ie, only those with good clinical responses remained in the study). When crisis rates were compared for the dropouts and for patients who
entered the study at the same time, but did not drop out, there was no difference between them.

All patients who completed the trial opted to continue therapy, and the frequency of reported crises decreased, but those suggestions of a beneficial effect are only suggestions. Previous attempts to lower the frequency of painful episodes in sickle cell anemia have all shown no effect of the study drug when compared with placebo. Until a carefully controlled and executed trial of HU is performed, questions of clinical efficacy and toxic/therapeutic ratio remain unanswered.

The same CCSCD study that suggested a beneficial effect of increased HbF level also showed increased frequency of painful attacks in patients with higher Hct. HU therapy increases Hb level (and Hct) to some degree, and also increases F/F cell. The two would have opposing effects on the viscosity of deoxygenated blood. Changes in Hb levels in our patients were not large, and our data suggest that they were more than offset by increases in percent F cells and F/F cell. The potential hazard remains, but would appear to be less than that posed by other attempts to raise HbF levels.

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