Hydroxyurea for Treatment of Severe Sickle Cell Anemia: A Pediatric Clinical Trial

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Hydroxyurea (HU) enhances the synthesis of fetal hemoglobin (HbF) and can improve the clinical course of some adult patients with sickle cell anemia (SCA). In a randomized trial, we studied the biologic effects and the clinical benefit of HU in children and young adults with severe SCA. Twenty-five patients (median age, 9 years) were randomized to receive either HU (at the initial dosage of 20 mg/kg/d) or a placebo for 6 months and were then switched to the other arm for the next 6 months. Among the 22 evaluable patients (median age, 8 years), significant increases in HbF and mean corpuscular volume occurred during the HU treatment period. The white blood cell and reticulocyte counts decreased significantly, but these changes were not clinically relevant.

The major problems in sickle cell anemia (SCA) are increased risk of severe infections during infancy and childhood and repeated vaso-occlusive crises that may lead to severe organ failure. Recurrences of painful episodes or evidence of organ dysfunction are clearly associated with a poorer prognosis and early mortality in adulthood.3 Hydroyxurea (HU) enhances the synthesis of fetal hemoglobin (HbF) and can improve the clinical course of some adult affected SCA patients, of whom 22 are evaluable.

MATERIALS AND METHODS

Twenty-five children and young adults severely affected by SCA were selected to receive HU. They all originate from Central African countries (24 from Zaire and 1 from Cameroon). There were 13 girls and 12 boys. Ages ranged from 2 to 22 years, with a median of 9 years. Because this was a pilot study, no sample size was predetermined. All patients eligible for the study were entered at the two participating institutions between June 1992 and December 1993.

These 25 patients were initially selected for the study on the basis of severe clinical disease (Table 1). To be eligible for the study, the patients had to have homozygous SCA. Patients with sickle cell β-thalassemia were excluded, but patients with α-gene deletion were included. To enter the study, patients had to have reported more than 3 vaso-occlusive crises in the year before entry into the study and/or have a previous history of stroke, acute chest syndrome, recurrent crises without free interval, or splenic sequestration. Transfusion therapy is clearly recommended in preventing recurrent stroke. Because many of our SCA patients develop alloimmunization, this therapy may become ineffective in them. For this reason, patients with a previous history of stroke were also eligible for the trial, but only if appropriate transfusion therapy could not be conducted (severe alloimmunization in 1 patient and refusal in another).

The study was approved by both hospital ethics committees and informed consent was obtained from the patient and/or the parents or tutors.

Patients and/or parents or tutors were made aware of the potential risk of teratogenesis and mutagenesis associated with chronic exposure to HU. Teenage and adult patients were counselled regarding contraception and pregnancy.

By drawing consecutive sealed envelopes, patients were randomly allocated to one of the following treatment sequences: either HU first for a period of 6 months, followed by placebo for 6 months, or placebo first, followed by HU for an additional 6 months. The study was run single-blind (the patient was unaware of the treatment received, but the physician had knowledge of the treatment). The drug or the placebo was provided monthly for each patient by the hospital pharmacy. Both were indistinguishable. The trial was run single-blind rather than double-blind because of the logistic difficulty of blinding the attending physician to the treatment received. Indeed, for the blinding to be effective, the attending physician should have been denied access to the pharmacy records and to the laboratory results in the hospital database.

HU was administered at an initial dosage of 20 mg/kg every day. If no change in HbF level had occurred after 2 months (increase <2%), the doses of HU were increased up to 25 mg/kg/d. In case of bone marrow toxicity defined by a white blood cell count (WBC) less than 3 × 10^9/L and/or a platelet count less than 80 × 10^9/L, the initial dosage was reduced by 50%. All patients received 1 mg of folic acid each day. Children under 6 years of age received oral penicillin in addition. All patients were seen monthly in the outpatient clinic.

The end points of interest in this study were the number of hospitalizations and the number of days in hospital.

Heparin was administered at an initial dosage of 20 mg/kg every day. If no change in HbF level had occurred after 2 months (increase <2%), the doses of HU were increased up to 25 mg/kg/d. In case of bone marrow toxicity defined by a white blood cell count (WBC) less than 3 × 10^9/L and/or a platelet count less than 80 × 10^9/L, the initial dosage was reduced by 50%. All patients received 1 mg of folic acid each day. Children under 6 years of age received oral penicillin in addition. All patients were seen monthly in the outpatient clinic.

The end points of interest in this study were the number of hospitalizations and the number of days in hospital.

The number of days of use was also initially considered as an endpoint of the trial. Patients or their parents were asked to fill out a card to report their painful episodes. However, during the course of the trial, it became clear that this information could not be reliably obtained from a majority of the patients, and this endpoint was dropped from all analyses.
Routine hematologic measurements were performed each month using an H1 hematology analyzer (Bayer Diagnostics, Tarrytown, NY). Renal and hepatic tests were also performed each month. HbF was quantitated by the alkali denaturation method when its level was less than 15% or by scanning after citrate agar gel electrophoresis when its level was greater than 15%.

The paired Student's t-test was used to determine the level of significance of differences between biologic parameters before and after HU treatment. Correlations were computed using the Pearson's correlation test.

The standard analysis of the 2-treatment 2-period cross-over trial was adopted. The Wilcoxon rank-sum test was used to assess the significance of the effects of treatment, period, and carry-over. Two-tailed P values less than .05 were considered to be statistically significant.

RESULTS

Twenty-five patients entered the study. Twenty-four patients fulfilled the initial inclusion criteria. One patient was included although he experienced only 2 vaso-occlusive crises during the 12 months preceding the study. Three patients were excluded after 4 to 5 months because they failed to attend the monthly evaluation visits. Because these patients could not be evaluated at the end of their first time period and during their second time period, they could not contribute to the treatment comparisons and were therefore excluded from all analyses. There were thus 22 patients who could be analyzed (median age, 8 years).

One patient has been chronically transfused since years before the trial for recurrent stroke and moyamoya syndrome, but chronic transfusion was stopped 3 months before entry into the trial because he developed severe alloimmunization.

The mean initial hemoglobin level was 8.1 g/dL (range, 6.7 to 9.3 g/dL). After 6 months of HU treatment, the mean hemoglobin level increased to 8.5 g/dL (range, 7.2 to 10 g/dL). An increase of more than 1 g/dL was observed in 9 of 22 evaluable patients (Table 2). The difference was not significant (P = .068).

The mean initial mean corpuscular volume (MCV) was 85.2 fl (range, 63 to 98.3 fl). After 6 months of HU therapy, it increased to 95.5 fl (range, 68 to 112 fl). This change was highly significant (P < .001; Table 2). The mean corpuscular hemoglobin concentration (MCHC) did not change significantly (P = .069).

The mean initial HbF value was 4.7% (range, 0.1% to 20.2%). After 6 months of HU therapy, it increased up to 15% (range, 1.1% to 38%). This difference was highly significant (P < .001). A threefold increase of the initial HbF value was observed in 11 patients; a twofold increase was observed in 7 patients. The increase of HbF correlated significantly with the increase of MCV (Fig 1; r = .5, P = .017). In some patients, the MCV increased weeks before any increase in the HbF level.

After 6 months of HU treatment, the reticulocyte count significantly decreased from 149% ± 54% to 103% ± 49% (P < .001).

The absolute neutrophil count remained within the normal range in all patients; however, it decreased significantly in patients treated with HU (Table 2). Only 2 children developed transient mild thrombocytopenia between 100 × 10⁹/L and 140 × 10⁹/L, which did not require a reduction of the initial HU doses. Five patients reached the final dose of 25 mg/kg/d. No other biologic side effect was observed. No patient required a dose reduction for thrombocytopenia or leukopenia.

No correlation was found between HbF increase and the initial HbF level, WBC count, or platelet count.

In addition to the significant changes in the MCV and HbF level, most patients responded favorably clinically. In 16 patients, a complete disappearance of vaso-occlusive events requiring hospitalization occurred within the first month of HU treatment. Among these 16 patients, 3 were severely disabled: 1 with a previous history of recurrent strokes, 1 with severe chronic bone pain, and 1 with 208 days in hospital during the year preceding entry into the trial.

No relevant change in the number of crises requiring hospitalization occurred in 6 patients. Of interest, in these 6 patients, the HbF level only slightly increased from 1.7% (range, 0.1% to 4.7%) to 3.2% (range, 1.1% to 7%). Three of them reached a dose increase up to 25 mg/kg according

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the 25 Patients</th>
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<tbody>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>No. of crises in the year before study</td>
</tr>
<tr>
<td>&lt;3</td>
</tr>
<tr>
<td>3-5</td>
</tr>
<tr>
<td>6-9</td>
</tr>
<tr>
<td>&gt;10</td>
</tr>
<tr>
<td>Complications of sickle cell anemia</td>
</tr>
<tr>
<td>Chest syndrome</td>
</tr>
<tr>
<td>Osteomyelitis/osteonecrosis</td>
</tr>
<tr>
<td>Stroke</td>
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<tr>
<td>Splenic sequestration</td>
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</tbody>
</table>

| Table 2. Mean Hematologic Values Before and After 6 Months of Treatment With HU |
|-----------------------------|---------------------------------|-----------------------------|-----------------------------|
|                          | Before HU Therapy (mean ± SD)  | After HU Therapy (mean ± SD) | P   |
| Hb (g/dL)                | 8.1 ± 0.75                     | 8.5 ± 0.83                  | NS |
| MCV (fL)                 | 86.2 ± 9.74                    | 95.5 ± 11.57                | .001|
| MCHC (%)                 | 33.0 ± 2.08                    | 32.3 ± 1.12                 | NS |
| Platelets (x10⁹/L)       | 443.2 ± 189.1                  | 386.7 ± 144.6               | NS |
| WBC (x10⁹/L)             | 12.47 ± 4.58                   | 8.90 ± 2.51                 | .001|
| HbF (%)                  | 4.65 ± 4.81                    | 15.34 ± 11.3                | .001|
| Reticulocytes (%)        | 148.6 ± 53.8                   | 102.7 ± 48.5                | .001|

Abbreviations: SD, standard deviation; NS, not significant.

* Paired Student's t-test.
to the initial protocol, whereas only 2 of the 16 responders needed a dose increase. Two of the three patients who were excluded from analysis remained highly symptomatic (recurrent acute chest syndrome [ACS] or/and high hospitalization rate for crises). The third patient, who had a history of previous stroke, died 2.5 years after inclusion from suspected intracranial hemorrhage.

Overall, the number of hospitalizations was reduced when patients were on HU therapy as compared with placebo. The statistical tests (Table 3) indicated a very significant effect of treatment and no significant period effect or carry-over effect from the first to the second 6-month period. Combining the results of both periods (Table 4), 16 patients of 22 (73%) did not require any hospitalization for painful episodes when treated with HU as compared with only 3 of 22 (14%) when treated with placebo.

The number of days in hospital was also significantly lower when patients were on HU therapy (range, 0 to 19 days) than when they were on placebo (range, 0 to 104 days). During the first 6-month period, the mean number of days in hospital was 5.3 days for HU, as compared with 15.2 days for placebo; during the second 6-month period, these figures were, respectively, 1.8 days for HU and 8.2 days for placebo (Fig 2). The treatment difference was statistically significant, but the downward trend from the first to the second period was not (Table 3).

No pregnancies occurred in the female patients.

**DISCUSSION**

Stimulating expression of the fetal globin genes has been suggested as a possible therapy for severe SCA. The clinical benefit of higher HbF levels is clearly shown by the reduction in the risk of stroke or acute chest syndrome.\(^\text{11}\) Furthermore,

\[
\text{MCV increment (fl)}
\]

\[
\begin{array}{cccc}
0 & 5 & 10 & 15 \\
\text{HbF increment (\%)} & 0 & 5 & 10 \\
\end{array}
\]

\[n=22\]

\[r=0.56\]

\[p<0.01\]

\[
\text{Table 3. } P \text{ Values of the Statistical Tests Performed on the Number of Hospitalizations and the Number of Days in Hospital}
\]

<table>
<thead>
<tr>
<th></th>
<th>Treatment Effect</th>
<th>Period Effect</th>
<th>Carry-Over Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of hospitalizations</td>
<td>(P = .0016)</td>
<td>(P = .47)</td>
<td>(P = .80)</td>
</tr>
<tr>
<td>No. of days in hospital</td>
<td>(P = .0027)</td>
<td>(P = .15)</td>
<td>(P = .64)</td>
</tr>
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**Table 4. Number of Hospitalizations and Number of Days in Hospital by Treatment (Both Periods Combined)**

<table>
<thead>
<tr>
<th></th>
<th>HU (N = 22)</th>
<th>Placebo (N = 22)</th>
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</thead>
<tbody>
<tr>
<td>No. of hospitalizations</td>
<td>(0)</td>
<td>16</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No. of days in hospital</td>
<td>(0-10)</td>
<td>16</td>
</tr>
<tr>
<td>(&gt;10)</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Range</td>
<td>(0-19)</td>
<td>4</td>
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<td></td>
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<td>0-104</td>
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\[
\text{Fig 1. Correlation between the increase of HbF and that of MCV. (□) Patients who responded clinically. (■) Patients who did not respond clinically.}
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\[
\text{Fig 2. Mean number of days in hospital by time period and treatment. One group of 12 patients started on placebo and switched to HU (solid line). The other group of 10 patients started on HU and switched to placebo (dotted line).}
\]
patients with high HbF levels develop less vaso-occlusive crises, need less hospitalization, and have a better life expectancy. Several studies have shown that HbF could be reliably stimulated in patients with SCA.\textsuperscript{12-14} The clinical benefit of this approach has remained, until recently, difficult to assess.\textsuperscript{15-17}

In a large published study\textsuperscript{14} in which some patients have been treated up for 110 weeks, a correlation was found between the initial Hb level, the initial HbF level, the initial WBC count, and the increase in the HbF level. In our studied population, there is a clear relationship between the increase in HbF level and the increase in MCV. However, we did not confirm the significant impact of initial HbF level, WBC count, initial Hb level, or initial MCV on the HbF response. On a population of sickle cell β-thalassemia patients on long-term therapy with HU, Voskaridou et al\textsuperscript{18} also showed a significant increase of MCV, MCHC, and HbF level. In our series of patients, a statistically significant marrow suppression occurred on HU, with a decrease in WBC and reticulocyte counts, but this effect was not clinically relevant.

In some patients, an increase in MCV occurred earlier than an increase in HbF level. No clear explanation has been proposed for the relative macrocytosis under HU exposure.\textsuperscript{19,20} In our patients, MCHC did not change significantly under HU exposure; thus, we cannot interpret larger MCV as a sign of better red blood cell hydration or membrane modification.

As far as clinical improvement is concerned, 73% of the evaluable patients responded favorably. They felt better and did not require any hospitalization during the trial period. This also occurred in previously seriously disabled patients.

In a double-blind, randomized placebo controlled trial including 299 symptomatic adult patients, an interim analysis of the data showed that the patients assigned to HU treatment had lower annual rates of crises than did the patients receiving placebo. Fewer patients assigned to HU had chest syndrome and fewer received transfusions.\textsuperscript{21} This led to an earlier termination of the trial.\textsuperscript{21} The clinical benefit of HU was also studied in a subset of sickle cell β-thalassemia patients; all 14 adult patients enrolled in the study improved on HU therapy and ceased having pain or other complaints.\textsuperscript{18}

In our randomized cross-over trial, treatment with HU resulted in a clear clinical benefit, with a significant reduction in the number of hospitalizations and in the number of hospitalization days. This is, as far as we know, the first report on HU efficacy in children and young adults.

In our series, no clinically relevant toxicity was associated with HU therapy. Our data support the use of HU therapy for severely affected children and young adults. Nevertheless, parents and patients should remain aware of the potential risks related to HU therapy, such as myelotoxicity or teratogenicity.

The safety of long-term HU administration, particularly with respect to leukemogenesis, should now be evaluated in children and young adults.\textsuperscript{22} Prospective trials are needed to assess the possible benefit of HU on the prevention of organ damage and, ultimately, on life expectancy.

ACKNOWLEDGMENT

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REFERENCES


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