Patients with hemoglobinopathies such as sickle cell anemia and thalassemia might be clinically improved if their levels of fetal hemoglobin could be augmented. It has been shown recently that certain drugs most often used for chemotherapy of malignancies may affect Hb F production. These drugs were tested initially in subhuman primates and subsequently in patients with hemoglobinopathies. The first agent used, 5-azacytidine, could lead to hypomethylation of genes, which may then be expressed, but it also has cell cycle activity. The second group of drugs, including cytosine arabinoside and hydroxyurea, are cycle active, without directly affecting DNA methylation. Both groups of drugs produced increased Hb F in baboons or monkeys that were made chronically anemic and had expanded erythroid compartments due to phlebotomies. 5-Azacytidine was then carefully administered to selected patients with sickle cell anemia and thalassemia and resulted in increased Hb F and total Hb levels. Recently, hydroxyurea was also reported to increase Hb F in a small number of patients with sickle cell anemia. Since the chemotherapeutic drugs may have carcinogenic or other toxic side effects, large clinical trials in patients without prior malignancies must be approached very cautiously. However, patients with such diseases are already receiving these drugs and might be a source of useful information regarding their effect on Hb F synthesis, albeit in patients lacking hemoglobin disorders. In the past four years, one of us (H.G.) has administered hydroxyurea when indicated clinically to a group of adults with myeloproliferative disorders. We examined the effect of this agent on fetal hemoglobin and other fetal red cell characteristics. The results of this analysis suggest that Hb F increases in some patients on hydroxyurea treatment without hemoglobinopathies or anemia. Careful analysis of the response of these patients may provide useful information regarding the administration of hydroxyurea in patients with hemoglobinopathies.

Materials and Methods

Since February 1980, 13 patients were treated with hydroxyurea for myelosuppression. There were five males and eight females, ranging in age from 26 to 82, with a median age of 66 years. The diagnoses included six polycythemia vera (PV), five polycythemia vera with myeloid metaplasia, one agnogenic myeloid metaplasia, and one chronic myelogenous leukemia (CML). Diagnoses were made based on history, physical examination, and laboratory tests, using criteria described previously. Two of the patients have been described elsewhere. The patients took 0.5 to 2 g of hydroxyurea daily, once or twice a day, with interludes of no treatment that were determined by their blood counts and clinical courses. This dosage is in the range of 7 to 40 mg/kg/d. A summary of the clinical parameters is shown in Table 1. "Responders" are defined as those patients whose Hb F increased during hydroxyurea treatment.

All procedures were approved by the Research Advisory Committee of the Mt Sinai School of Medicine. Hematologic data were obtained by standard methods. Blood anticoagulated with EDTA was used to determine the percentage of Hb F by alkali denaturation, and the percentage of Hb A2 by column chromatography (Isolab, Akron, Ohio), as well as the red cell size histogram using a Coulter C-1000 Channelizer (Coulter Electronics, Hialeah, Fla) and ZBI counter. The coefficient of variation (CV) was determined as the SD/mean, following log transformation as described previously. The percentage of F cells was measured by the acid elution slide test, using a kit from Boehringer Mannheim Diagnostics, Houston. This method is somewhat insensitive and imprecise, but usually correlates with the percentage of Hb F. The red cell titer of i antigen was determined by agglutination with fivefold dilutions of anti-i (Den), kindly provided by Marie Crookston, Toronto General Hospital. Normal values for the tests used include: Hb F, <1%; A2, 1.5% to 3.5%; CV, 13.3% to 16.5%; F cells, ≥1%; and i titer, 0.

The data presented here were obtained during long-term clinical management of the patients' myeloproliferative diseases; not all patients were followed closely for all parameters. Specifically, the percentage of Hb F was measured on the average at three- to 11-week intervals (mean, six weeks) in the responders, and two- to 30-week intervals (mean, 12 weeks) in the nonresponders. Since the analysis was ongoing, the finding of increased Hb F often resulted in a closer follow-up. The results thus provide a minimal estimate of the effect of hydroxyurea on Hb F and erythropoiesis in this group of patients.

From the Polly Annenberg Levee Hematology Center, Departments of Medicine and Pediatrics, Mt Sinai School of Medicine, New York.

Supported in part by grants from the National Institutes of Health (No. HL26132, CA31656, and RR71), the March of Dimes/Birth Defects Foundation (No. 6-386), the Jack Martin Fund, and an Irma T. Hirschl Trust Career Scientist Award (to B.P.A.).

Submitted Oct 1, 1984; accepted Feb 14, 1985.

Address reprint requests to Dr Blanche P. Alter, Division of Hematology, Mt Sinai School of Medicine, One Gustave L. Levy Pl, New York, NY 10029.

© 1985 by Grune & Stratton, Inc.

www.bloodjournal.org From www.bloodjournal.org by guest on November 15, 2017. For personal use only.
Table 2. Results of Hydroxyurea Treatment

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Sex</th>
<th>Date of Diagnosis</th>
<th>Previous Treatment</th>
<th>Date of HU Treatment</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>PV</td>
<td>69</td>
<td>F</td>
<td>1949</td>
<td>C</td>
<td>9/10/80</td>
<td>Died 3/82</td>
</tr>
<tr>
<td>4</td>
<td>AMyM</td>
<td>58</td>
<td>M</td>
<td>1979</td>
<td>C</td>
<td>10/13/80</td>
<td>Splenectomy 1981</td>
</tr>
<tr>
<td>11</td>
<td>PV-MyM</td>
<td>64</td>
<td>F</td>
<td>1978</td>
<td>Phleb</td>
<td>1/27/82</td>
<td>On HU</td>
</tr>
<tr>
<td>Nonresponders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>PV</td>
<td>50</td>
<td>M</td>
<td>1978</td>
<td>32P</td>
<td>2/10/80</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>PV-MyM</td>
<td>66</td>
<td>M</td>
<td>1972</td>
<td>M</td>
<td>10/13/80</td>
<td>Died 3/82</td>
</tr>
<tr>
<td>6</td>
<td>CML</td>
<td>26</td>
<td>F</td>
<td>1976</td>
<td>CCNU, Ara C, HU</td>
<td>10/15/80</td>
<td>Died 11/83</td>
</tr>
<tr>
<td>7</td>
<td>PV-MyM</td>
<td>68</td>
<td>F</td>
<td>1960</td>
<td>B</td>
<td>10/15/80</td>
<td>Died 7/83</td>
</tr>
<tr>
<td>9</td>
<td>PV</td>
<td>82</td>
<td>M</td>
<td>1968</td>
<td>B, C</td>
<td>4/28/81</td>
<td>Died 6/83</td>
</tr>
<tr>
<td>10</td>
<td>PV</td>
<td>74</td>
<td>F</td>
<td>1976</td>
<td>Phleb</td>
<td>4/29/81</td>
<td>No treatment, basal cell cancer</td>
</tr>
<tr>
<td>12</td>
<td>PV</td>
<td>68</td>
<td>M</td>
<td>1972</td>
<td>C, M</td>
<td>4/20/82</td>
<td>No treatment</td>
</tr>
<tr>
<td>13</td>
<td>PV-MyM</td>
<td>63</td>
<td>F</td>
<td>1970</td>
<td>Phleb</td>
<td>8/24/82</td>
<td>On HU, 0.5 g</td>
</tr>
</tbody>
</table>

Cases are numbered according to accrual sequence. Patients with PV or PV-MyM all had phlebotomies in the past. PV, polycythemia vera; MyM, myeloid metaplasia; AMyM, agnogenic myeloid metaplasia; CML, chronic myelogenous leukemia; B, busulfan; C, chlorambucil; M, melphalan; phleb, phlebotomy; Ara C, cytosine arabinoside; HU, hydroxyurea; NA, information not available, but patient alive.

RESULTS

Responders. The Hb F level rose in four of the 13 patients (31%) who received hydroxyurea during the management of their myeloproliferative disorder (Table 2). These four included the one with AMyM, two of the six with PV, and one of the five with PV-MyM. The duration of hydroxyurea administration ranged from 24 to 145 weeks. The time courses of changes in several hematologic parameters are shown in Figs 1 through 4.

The most rapid response was in patient No. 4 (Fig 1), who had AMyM. The Hb F had increased by two weeks of treatment and reached a plateau of approximately 5% by three weeks. This level was maintained for 17 weeks, during almost continuous treatment with 1 g of hydroxyurea per day. When hydroxyurea was administered intermittently, the level of Hb F declined to 1% and remained low thereafter. Initially, the percentage of F cells by the acid elution test rose from 4% to 9%, and then fell when the hydroxyurea schedule changed. The MCV rose slightly, but the maximum value was only 85 fl. Hydroxyurea administration did result in mild depression of WBCs and platelets, but not below normal values. The Hb actually rose, perhaps associated with a decrease in hypersplenism as the spleen shrank from 13 to 7 cm. No transfusions were required until after hydroxyurea was discontinued.

Patient No. 3 also showed a dramatic response (Fig 2). This patient had PV, requiring five phlebotomies of 350 mL during the year prior to hydroxyurea treatment. The last phlebotomy was 12 weeks before hydroxyurea administration. As soon as hydroxyurea was begun, the level of F cells began to rise and reached peak levels of 17% and 20% at ten weeks.
and 39 weeks, respectively. The percentage of Hb F rose at eight weeks, was above 8% at 12 and 53 weeks, and remained above 2% between eight and 65 weeks. The MCV began to rise immediately; red cell size histograms showed a bimodal pattern, with a distinct macrocytic population by eight weeks (Fig 5). This macrocytic population completely replaced the PV microcytes by 17 weeks. This interval, equivalent to one red cell survival time, suggests that only macrocytes were produced from the moment hydroxyurea treatment began. The patient’s leukocytosis and thrombocytosis were alleviated, but her polycythemia still required five phlebotomies in the year during hydroxyurea administration. There was no relationship between phlebotomy and Hb F. Her spleen decreased from 9 to 3 cm. As in the previous case, the effect of hydroxyurea on Hb F was more dramatic when therapy was continuous rather than intermittent.

Data for patient No. 11, a patient with PV-MyM, are shown in Fig 3. The percentage of Hb F was 0.1% and MCV was 75 fl prior to hydroxyurea treatment. After one year of continuous therapy, Hb F was 8%, and MCV was 96 fl. Following cessation of hydroxyurea treatment, the Hb F and MCV both declined to <1% and <80 fl, respectively, by 17 weeks, a single red cell life span. It is possible that no new red cells containing Hb F were produced from the moment hydroxyurea was discontinued. The desired effects of reduction of WBCs and platelets had been achieved rapidly, whereas reticulocyte and Hb counts actually rose during hydroxyurea (and iron) treatment. Phlebotomy was not required. The spleen decreased from 14 to 3 cm during treatment. This patient was then treated with 0.5 g of hydroxyurea per day for three months, with no increase in Hb F. However, when the dosage was raised to 0.75 to 1 g/d, Hb F again rose to 4%.

The response of patient No. 2, an individual with PV, was complex (Fig 4). The patient’s blood was monitored closely by acid elution for 73 weeks, at which time the F cell level had reached 12%, but the Hb F by alkali denaturation had not exceeded 2%. At the next visit (week 78), Hb F was 5% and remained elevated until hydroxyurea was discontinued.

Fig 1. Time course of hematologic parameters during treatment with hydroxyurea in patient No. 4, a 58-year-old male with agnogenic myeloid metaplasia. I Transfusion. (A) —- Hydroxyurea dosage (g/d); —- Hb F percentage. (B) —- F cell percentage; —- MCV (fl). (C) —- Hb (g/dL); —- reticulocyte percentage. (D) --- WBC count x 1,000 per cubic millimeter; —- platelet count x 1,000 per cubic millimeter.

Fig 2. Time course of hematologic parameters during treatment with hydroxyurea in patient No. 3, a 69-year-old female with polycythemia vera. Symbol “e” as in Fig 1. I Phlebotomy.
after 145 weeks of treatment. Hb F then decreased to <1% by nine weeks off therapy. The MCV rose from the beginning, although bimodality was not apparent on the red cell histogram until 72 weeks, the point at which the Hb F was also beginning to increase. The MCV then increased dramatically, reaching a peak of 118 fL at 122 weeks. It is important to note that hydroxyurea treatment was intermittent for the first 70 weeks, after which it was given continuously. Production of WBCs and platelets was decreased on hydroxyurea, as desired. Reticulocyte and Hb levels remained elevated, and a total of three phlebotomies was required during the 2.8 years of hydroxyurea treatment. The spleen size decreased from 15 to 6 cm. This patient later received a 12-week course of busulfan, which did not lead to increased Hb F or MCV.

Two of the four responders, patients No. 3 and 4 (persons with PV and AMyM, respectively), had increases in Hb F from 1.5% to >4% during treatment with chlorambucil in the year preceding the hydroxyurea therapy. Patient No. 2 had no measurement of Hb F during chlorambucil treatment, whereas patient No. 11 had an Hb F level of 0.25% during phlebotomy alone. By contrast, only one of the nine nonresponders had increased Hb F before hydroxyurea treatment. This was patient No. 6 (a patient with CML), who received cytosine arabinoside + CCNU, and whose Hb F was 2.5%.

![Fig 3. Time course of hematologic parameters during treatment with hydroxyurea in patient No. 11, a 64-year-old female with polycythemia vera that evolved into myeloid metaplasia before treatment with hydroxyurea. Symbols are as in Fig 1. (panel B). oral iron.](image)

![Fig 4. Time course of hematologic parameters during treatment with hydroxyurea in patient No. 2, a 57-year-old female with polycythemia vera. Symbols are as in Fig 1. (panel C). Phlebotomy: --, busulfan: — (panel C), oral iron.](image)
All of the other nonresponders had an Hb F level of <1% on a variety of treatment regimens.

**Nonresponders.** Data from the nine nonresponders are compared with those from the responders in Table 2. Hydroxyurea treatment was administered for 14 to 146 weeks, and Hb F was measured intermittently during this time. As the close monitoring of patient No. 2 shows (Fig 4), Hb F did not always increase immediately, and in fact, took a year and a half in that patient, in whom it rose after hydroxyurea was given continuously. In the responders, the average daily dosage of hydroxyurea ranged from 0.74 to 1.19 g (10 to 22 mg/kg/d), whereas this range was from 0.5 to 1.36 in the nonresponders (8 to 34 mg/kg/d). In the latter group, patients No. 1, 9, and 13 received a lower average daily dose of hydroxyurea than any of the responders (although only patient No. 1 received a lower dose per kilogram). In addition, treatment of the nonresponders appeared to be more intermittent, with one or more nontreatment weeks interspersed among treatment weeks. Three of the four responders received hydroxyurea for at least 34 consecutive weeks, whereas only three of the nine nonresponders were so treated (patients No. 9, 12, and 13). Two of those took only 0.5 and 0.69 g/d. In fact, only a single nonresponder, patient No. 12, received continuous treatment comparable in both dosage (0.99 g/d) and duration (37 weeks) to the majority of the responding group. One nonresponder, patient No. 1, never received more than four weeks of continuous treatment.

Although F cells did rise to above 5% in nonresponder patients No. 7 and 10, Hb F levels were not significantly increased. In patient No. 7, the F cells disappeared at 12 weeks, when hydroxyurea therapy was switched from continuous to intermittent. In patient No. 10, hydroxyurea was discontinued after 14 weeks just as the percentage of F cells began to rise. Thus, more intensive administration of hydroxyurea to these two patients might have led to increased Hb F, but it was not clinically indicated.

The MCVs rose from <75 to >80 fl in all patients and were above 90 fl in eight of the nine nonresponders. This macrocytic response occurred even in patients with PV who did not receive iron but was unrelated to changes in Hb F. The titer of i antigen was increased in three of the four responders and decreased during hydroxyurea treatment, but not in relation to changes in MCV or Hb F. This membrane antigen titer was increased in only two of the nine nonresponders (patients No. 1 and 6, a PV patient previously treated with 32P, and CML, respectively) and remained elevated on hydroxyurea. The desired suppression of WBCs and platelets occurred uniformly, without clinically significant marrow toxicity. The Hb levels were not always suppressed, and phlebotomy was still required in some of the PV patients. Spleen size decreased in all four responders, and in three of the five nonresponders who had significant splenomegaly at the onset.

**DISCUSSION**

Our data indicate that hydroxyurea treatment leads to increased Hb F in some patients. Unlike patients or animals previously reported by others, our subjects were generally not anemic, nor did they have specific abnormalities of their beta globin genes which might be associated with increased gamma chain synthesis. However, our patients with myeloproliferative disease and those with hemoglobinopathies do share the feature of an expanded erythroid compartment. Other factors that may influence Hb F production include genetics, underlying disease, treatment, and marrow suppression and recovery.

A genetic predisposition to produce Hb F in response to erythroid stress can be identified using sensitive immunologic tests for F cells. Using less sensitive techniques, all of our patients had "normal" Hb F levels at the time hydroxyurea treatment began. Two of the four responders did have increased Hb F (to 4%) during previous treatment with chlorambucil, and 1% Hb F at the onset of our study, which was perhaps higher than the 0.2% Hb F found in the other two responders and all nonresponders. Thus, genetic factors might be involved in changes in Hb F in some patients receiving hydroxyurea.

Approximately 10% of patients with PV have been reported to have increased Hb F, although one of the PV patients with increased Hb F was on busulfan, and two were on alkalyating agents. Increased Hb F has also been reported in 10% to 20% of patients with other types of myeloproliferative syndromes, but the exact relationship of this finding to the disease itself or to past or concurrent chemotherapy is difficult to discern.

Although Hb F sometimes increases in patients who receive chemotherapy, more often it rises during recovery from transient marrow suppression due to chemotherapy, following bone marrow transplantation, or during autologous recovery from aplastic anemia or red cell aplasia. In these patients, Hb F usually reaches its peak in eight weeks, but elevated levels often persist for more than six months.
The red cells of these patients demonstrate other features of 'fetal-like erythropoiesis,' such as macrocytosis and increased levels of i antigen.

Fetal-like erythropoiesis was not a prominent feature of our series. Macrocytosis certainly developed, but can be explained by interference with cell division during treatment with an agent that inhibits DNA synthesis. Some patients had red cells with increased titers of i antigen before hydroxyurea began, perhaps related to the dyserythropoiesis of myeloproliferative disease. These levels did not increase during treatment, nor did i antigen appear in patients in whom it was not already present. There was no relation among i antigen, MCV, and Hb F response. The Hb F response occurred only during hydroxyurea administration. When hydroxyurea was discontinued, or switched from a continuous to an intermittent schedule, Hb F decreased immediately and was gone within a single normal red cell life span. Thus, there was no fetal-like erythropoiesis during recovery from drug-induced marrow suppression.

A hydroxyurea schedule of approximately 15 to 20 mg/kg/d sufficed to increase Hb F without producing serious marrow toxicity. Patients with hemoglobinopathies, in whom marrow suppression is not therapeutically desirable, currently receive 50 mg/kg/d three to five days per week. It is possible that the slightly lower but continuous dose used in the patients with myeloproliferative disease would be equally effective in increasing Hb F without producing marrow suppression in those with hemoglobinopathies.

How does hydroxyurea increase Hb F? Patients with myeloproliferative disease and those with hemoglobinopathies have expanded erythropoiesis and erythroid stem cells that are sensitive to very low concentrations of erythropoietin in vitro. Normal erythropoiesis derives predominantly from self-renewal of rapidly dividing proerythroblasts or their immediate precursors, with <10% input from earlier noncycling progenitor cells. Hydroxyurea might eliminate the dividing mature erythroid precursors, leading to increased differentiation from the earlier progenitors and immature BFU-E that have not lost the propensity to produce some Hb F and do not undergo the normal maturational loss of their Hb F program. During continuous hydroxyurea treatment, substantially >10% of the erythroid compartment might be derived from this earlier progenitor cell pool, with the only hemoglobin program expressed by these cells thus being one for increased Hb F.

Administration of hydroxyurea to patients with diseases other than hemoglobinopathies provides an ethically acceptable model group for investigation into the role of cycle active drugs in increasing Hb F production. Long-term follow-up of such patients will provide information regarding possible toxicities of such agents. For example, the Polycythemia Vera Study Group has treated approximately 100 patients with hydroxyurea for more than three years with no evidence of a leukemogenic effect (P.D. Berk, Mt Sinai School of Medicine, personal communication, September 1984). Careful scrutiny of the treatment schedules may permit selection of a dosage regimen that will also increase Hb F in patients with hemoglobinopathies. Investigation of the cell biology of erythropoiesis and Hb F production during treatment with cycle active drugs may lead to further understanding of the normal switch from fetal to adult hemoglobin and of Hb F regulation in adults.

REFERENCES

18. Alter BP, Rappaport JM, Huisman THJ, Schroeder WA,
33. Alpen EL, Cranmore D: Cellular kinetics and iron utilization in bone marrow as observed by Fe59 radioautography. Ann NY Acad Sci 77:753, 1959
The effect of hydroxyurea on hemoglobin F in patients with myeloproliferative syndromes

BP Alter and HS Gilbert