Induction of Fetal Hemoglobin Production in Subjects With Sickle Cell Anemia by Oral Sodium Phenylbutyrate

By George J. Dover, Saul Brusilow, and Samuel Charache

Intravenous arginine butyrate has been shown to increase fetal hemoglobin (HbF) in sickle cell and thalassemia patients. Recently, we observed that sodium 4-phenylbutyrate, a drug administered orally to treat urea cycle disorders, increases HbF production in nonanemic children and adults. We treated six subjects with sickle cell disease over a period of 14 to 179 days. All subjects received their initial therapy of 9 to 13 g/m²/day as 0.5-g tablets of sodium 4-phenylbutyrate as inpatients. All subjects showed a rapid increase in the percentage of F-reticulocytes (pretreatment, 1% to 20%; posttreatment, 10% to 44%). Four subjects were treated only 11 to 25 days as inpatients. Two of these four subjects failed to respond to the outpatient component because of their inability to maintain an intake of 30 to 40 tablets per day.

Because fetal hemoglobin (HbF) interferes with the polymerization of sickle hemoglobin (HbS), increases in HbF production could decrease the severity of disease in subjects with sickle cell anemia (SS).

In a recent study of the clinical course of SS disease, the frequency of painful crises appeared to be inversely related to HbF levels greater than 4%.

Two categories of drugs have now been shown to increase hemolysate HbF levels in SS patients: cytotoxic agents that interfere with DNA synthesis (5-azacytidine and hydroxyurea) and hematopoietic growth factors (erythropoietin). A third class of drug, aliphatic butyrate salts, has been shown to increase HbF production in animals and in cultures of human erythroid progenitors. It has long been known that sodium butyrate can induce erythroid differentiation in MEL cells and in K562 cells. It has also been noted that butyrate can induce ‘‘switching’’ from adult to embryonic hemoglobin production (HbF) in an avian model. Recently, Perrine et al have shown that intravenous infusions of arginine butyrate transiently increase HbF production in subjects with both sickle cell disease and thalassemia intermedia. Until now, no orally administered butyrate salt has been used to treat subjects with sickle cell disease, and no subject has been treated for more than a 2- to 3-week period.

Sodium phenylbutyrate is an investigational drug currently used in a Food and Drug Administration-approved phase III trial of its use in the treatment of urea cycle disorders. After administration, it is beta oxidized to phenylacetate, which is then conjugated with glutamine to yield phenylacetylglutamine, the two nitrogen atoms of which substitute for the two nitrogen atoms of the defective urea pathway as a means for urinary excretion of waste nitrogen products. There have now been more than 100 patient years of experience with this drug in urea cycle disorders and no untoward effects have been shown at the recommended doses (9.0 to 13.0 g/m²/day). The principal problem has been compliance, because the amount of drug required each day is quite high; the adult dose is approximately 20 g/d (40 0.5-g tablets).

Phenylacetate has been shown by Samid et al to induce differentiation in K562 cells. At the suggestion of Samid, Brusilow and Dover noted that patients with urea cycle disorders treated with sodium phenylbutyrate had increased F-cell production. In this report, we describe the effect of orally administered sodium phenylbutyrate in five subjects with sickle cell anemia. All five subjects had rapid increases in the percentage of HbF-containing reticulocytes (F- reticulocytes). Two subjects treated for 178 and 154 days had increases in hemolysate HbF levels of 7.4% and 5.6%. As expected, treatment was not associated with bone marrow depression.

MATERIALS AND METHODS

The study was approved by the Joint Committee on Clinical Investigation of The Johns Hopkins Medical Institutions, and written informed consent was obtained from all subjects. All study participants had sickle cell anemia and were, or had been, patients at this hospital (see Tables 1 and 2 for pretreatment data). All patients were hospitalized for initial studies in the Adult or Pediatric Inpatient Johns Hopkins Hospital Clinical Research Centers; outpatient studies were followed in the Adult Outpatient Clinical Research Center.

The maximal dose of sodium phenylbutyrate administered to adult patients with urea cycle disorders (13 g/m²/day) was administered in three divided doses with meals as 500-mg tablets. Subjects A, B, and D received two 8- to 10-day courses with an intervening period during which therapy was discontinued; treatment was restarted and continued after discharge, but subject B was heavily transfused at another hospital shortly after discharge, precluding interpretation of subsequent hematologic data. Subject C’s therapy was discontinued after he developed a rash at 135 days. Subject D was treated for 43


From the Departments of Pediatrics, Medicine and Laboratory Medicine, Johns Hopkins University Medical School, Baltimore, MD.

Submitted October 14, 1993; accepted March 16, 1994.

Supported in part by National Institutes of Health Grants No. HL 28028 (G.J.D.), HD 11134, and HD 26358 (S.B.), by Clinical Research Center Grants No. RR-0035 and RR-00722, and by Pediatric Clinical Research Unit No. 5-M01-R00052.

Address reprint requests to George J. Dover, MD, Ross Research Bldg, Room 1125, 720 Rutland Ave, Baltimore, MD 21205.

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RESULTS

HbF production. Figure 1 indicates the rapid increase in the percentage of F-reticulocytes seen in all six subjects during the first 10 days of inpatient treatment. The increase and decrease in F-reticulocyte production in subjects A, B, and D, as inpatient treatment was started, stopped and re-started, suggested that increases in F-reticulocyte production were attributable specifically to sodium phenylbutyrate therapy. Figure 2 and 3: We have previously shown that the percentage of F-reticulocyte levels do not change over time.26 The rapidity of the increases in F-reticulocyte levels is comparable with that seen in SS patients treated with 5-azacytidine. The variability in the magnitude of the response to short-term treatment (<10 days) is similar to that seen with both 5-azacytidine and hydroxyurea (HU).10,27

Table 2 compares pretreatment levels with peak levels of F-reticulocytes, F cells, and hemolysate HbF in all six subjects. All subjects showed an increase in F-reticulocyte production. Subjects A and F, treated for 178 and 154 days, respectively, showed an increase in HbF to levels greater than 15%. Changes in F cells and HbF in subject B are somewhat difficult to interpret because of prior transfusions. Figure 3 shows changes in HbF, the percentage of F-reticulocytes, and the percentage of F cells in subject A and F during treatment in greater detail.

Other changes noted during treatment. Marrow suppression was not seen in any subject. In particular, after 179 days of treatment, subject A showed no indication of change in hemoglobin (9.3 to 10.5 g/dL), white blood cell count (12.5 to 10.5 x 10^9/L), platelet count (249 to 313 x 10^9/L), absolute neutrophil count (5.4 to 6.3 x 10^9/L), reticulocyte count (8.6% to 8.5%), mean corpuscular volume (MCV; 105 to 108 fl), MCHC (35 to 35 g/dL), or dense cells (17% to 16%). Patient F also showed no changes in these parameters.
PHENYLBUTYRATE INCREASES HbF IN SS DISEASE

Subject A, after approximately 14 days of therapy, developed ankle edema. This was associated with an increase in weight of approximately 5 kg. The edema cleared with a few days of hydrochlorothiazide treatment, but has recurred transiently. His weight gain continued and is now maintained at 4 to 5 kg more than baseline. Edema was not noted in any other subject; however, subjects B, C, and F also showed increases in weight during therapy. Increases in weight were not associated with changes in blood pressure or electrolyte abnormalities.

After 11 days of treatment, subject C developed a follicular pruritic rash over the arms and legs extending to the trunk. This persisted for approximately 14 days after the cessation of therapy. A dermatology consultant attributed the rash to either a drug reaction or to a transient viral illness. Subject F developed a fever between days 10 and 21 of therapy. No etiology was determined, sodium phenylbutyrate was not discontinued, and no reoccurrence of fever was seen while on outpatient therapy.

Plasma levels of phenylbutyrate and its products. Figure 4 shows changes in serum levels of phenylbutyrate, phenylacetate, and phenylacetylglutamine in subject A during a typical day of treatment. Similar patterns were seen in subjects B, D, and E. None of these subjects had detectable fasting morning levels of phenylacetate. However, subject C had a fasting morning plasma, phenylacetate level of 1.0 mmol/L. When the dose of sodium phenylbutyrate was reduced by approximately 50% in subject C, the fasting morning phenylacetate level was 0.5 mmol/L. Serial fasting morning plasma levels in subject C showed no incremental accumulation of phenylacetate.

The range of peak daytime plasma levels (in millimoles per liter) of the drug and its reaction products were phenylbutyrate, 1.1 to 1.95; phenylacetate, 0.57 to 1.72; and phenylacetylglutamine, 0.28 to 2.24. Plasma amino acid concentra-
tions were within normal limits and were unchanged during therapy with phenylbutyrate. In particular, plasma glutamine levels were maintained within normal limits.

**DISCUSSION**

**Mechanism of the increase in F-reticulocyte production.** At the present time, the drug most widely used on an experimental basis to increase fetal hemoglobin in SS patients is HU. A phase I/II trial of HU showed that HbF levels increased in 32 patients from a mean of 4% to 15%.27 A multicenter controlled clinical trial is underway to determine whether HU can decrease the crisis attack rate in SS patients. In the phase I/II trial, at the maximal tolerable dose, HU-treated patients showed a reduction in white blood cells by 40%, in platelets by 20%, and in reticulocyte counts by 40%, suggesting a connection between marrow depression and increased HbF production. One mechanism by which cytotoxic drugs (HU and 5-azacytidine) have been presumed to increase HbF is by inhibiting division of late erythroid precursors, with subsequent recruitment of earlier progenitors programmed to produce higher levels of HbF.29 Rapid induction of F-reticulocyte production (48 hours after beginning therapy), first noted with 5-azacytidine therapy29 and seen variably during induction with HU therapy,3,6,27,30 would not support that hypothesis. Both 5-azacytidine29 and HU30 have been shown to increase HbF production by a direct effect on late erythroid precursors. Absence of cytotoxicity and the extremely rapid onset of new F-reticulocyte production suggest that butyrate analogues act directly on late erythroid precursors, without any reduction in the number of early erythroid cells. These findings agree with in vitro experiments indicating direct induction of HbF in late erythroid precursors by sodium butyrate analogs.11 The fact that, in K562 cells, HU and sodium phenylacetate are synergistic in induction of erythroid differentiation suggests that these drugs may have different mechanism of action.31

**Potential antisickling effect of phenylbutyrate.** If HU therapy does decrease the sickle crisis attack rate, it is not clear whether such an effect can be attributed to HbF alone. HU therapy is associated with an increase in MCV, and although MCHC does not change, the number of dense (and light) cells does decrease.6,24 Sodium phenylbutyrate does not affect either MCV, MCHC, or the percentage of dense cells and may well affect HbS polymerization differently.

**Side effects of phenylbutyrate.** Because of its dose-limiting myelotoxicity6,23,27 and uncertain carcinogenic or mutagenic effects,32 HU has not yet been evaluated in the treatment of children with sickle cell disease. Data already collected in patients with urea-cycle disorders make those risks unlikely with sodium phenylbutyrate.

The increase in weight we observed might be assumed to be caused by an increase in body sodium and water. Twenty grams per day of sodium phenylbutyrate contributes 2.4 g of sodium (equivalent to 6.2 g of sodium chloride) to the diet (normal adult diet, 3 to 5 g/d). Only subject A exhibited any edema. Weight gain was seen in a number of patients receiving chronic HU therapy, and may be caused by decreased energy requirements secondary to a lowered hemolytic rate.27 It is unlikely that a similar mechanism can be invoked for sodium phenylbutyrate because no increase in hematocrit or in reticulocyte counts was seen. Subject C developed a skin rash that might have been related to the persistence of phenylacetate in his blood. Approximately 1 in 10 patients with urea-cycle disorders also showed some persistence of overnight fasting plasma phenylacetate levels with no evidence of dermatologic pathology.19,20

A major drawback to the use of phenylbutyrate is the high dose. The usual adult dose (20 g) requires taking 40 0.5-g tablets. Although the number of tablets will be less in children, this represents a significant problem in use and patient compliance with a therapeutic regimen. The drug in powder form has a bitter taste that, despite many attempts, cannot be disguised. Two of the three subjects treated after discharge from the hospital reported inability to maintain compliance. However, if clinical efficacy could be shown, the ingenuity of pharmaceutical manufacturers might surmount this problem.

**Future developments.** Careful dose-response analysis must be performed. Combination of phenylbutyrate and HU remains an interesting possibility. A synergistic or additive effect of this combination of drugs might increase the maximal HbF response or reduce the amount of HU necessary to induce a clinical significant level of HbF. Indeed, Fibach et al19 have recently reported synergistic effects of sodium phenylbutyrate and HU in K562 differentiation. On the basis of these preliminary reports, phase I/II trials have been started to further evaluate the efficacy and safety of sodium phenylbutyrate in patients with sickle cell disease and thalassemia.

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GJ Dover, S Brusilow and S Charache