

# Effect of hydroxyurea on G gamma chain fetal hemoglobin synthesis by sickle-cell disease patients

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## Abstract

Hydroxyurea is used for sickle-cell disease patients in order to increase fetal hemoglobin synthesis and consequently decrease the severity of pain episodes. Fetal hemoglobin, which is formed by  $\gamma$ -globin chains A and G, is present in a constant composition throughout fetal development: about 75% of  $G\gamma$  and 25% of  $A\gamma$ . In contrast, adult red cells contain about 40% of  $G\gamma$  and 60% of  $A\gamma$ . In the present study, we analyzed the effect of hydroxyurea induction on the  $\gamma$  chain composition of fetal hemoglobin in 31 sickle-cell disease patients treated with hydroxyurea. The control group was composed of 30 sickle-cell disease patients not treated with hydroxyurea in clinical steady state. The patients were older than 13 years and were not matched for age. All patients were seen at Hemocentro/UNICAMP and Boldrini Infante Center, Campinas, SP, Brazil. The levels of total hemoglobin were significantly higher in patients treated with hydroxyurea (mean  $\pm$  SD,  $9.6 \pm 2.16$  g/dl) than in untreated patients ( $8.07 \pm 0.91$  g/dl). Fetal hemoglobin levels were also higher in treated patients ( $14.16 \pm 8.31\%$ ) than in untreated patients ( $8.8 \pm 4.09\%$ ), as was the  $G\gamma/A\gamma$  ratio ( $1.45 \pm 0.78$  vs  $0.98 \pm 0.4$ ,  $P < 0.005$ ). The increase in the  $G\gamma/A\gamma$  ratio in patients treated with hydroxyurea suggests the prevalence of a pattern of fetal hemoglobin synthesis, whereas patients not treated with hydroxyurea maintain the adult pattern of fetal hemoglobin synthesis. Because no correlation was observed between the  $G\gamma/A\gamma$  ratio and total hemoglobin or fetal hemoglobin levels, the increase in  $G\gamma$  chain synthesis may not imply a higher production of hemoglobin.

## Key words

- Gamma chain
- Fetal hemoglobin
- Hydroxyurea
- Sickle-cell disease

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Fetal hemoglobin (HbF) interferes with the intracellular polymerization of sickle hemoglobin and has beneficial effects on patients with sickle-cell disease (SCD) (1). On this base, several agents such as 5-azacytidine and hydroxyurea (HU), which are known to stimulate HbF synthesis, have been used in SCD patients to decrease the frequency and

severity of pain episodes (2,3).

Previous studies showed that approximately 60% of the patients treated with HU responded with an increased HbF level and a reduction (44%) in the annual rate of painful episodes. There was also a significant variation in the ability of patients to respond to HU. Prolonged treatment is generally re-

quired to obtain the clinical response (4,5).

HbF is formed by  $\gamma$ -globin chain A or G according to the amino acid at the 136 position in the  $\gamma$  chain, i.e., alanine or glycine. The proportion of  $G\gamma$  to  $A\gamma$  is constant throughout fetal development: about 75% of  $G\gamma$  and 25% of  $A\gamma$ . However, in adult red cells, the small amount of HbF is composed mainly of  $A\gamma$  (60% vs 40% of  $G\gamma$ ) (6).

Sickle-cell anemia patients present different levels of HbF and a relation of HbF and  $G\gamma$  levels with the  $\beta^S$  haplotypes has been demonstrated. Senegal and Benin haplotypes

differed in  $G\gamma$  expression, mean percentage of HbF, which was higher in the Senegal than in the Benin type, while the Bantu haplotype had intermediate features (7). More recently, mRNA analysis strengthened these data. Sickle-cell anemia patients with Senegal and Saudi Arabia-India haplotypes had higher percentages of  $\gamma$  mRNA than Benin, Bantu or Cameroon types (8).

Various *in vitro* cell culture systems have been used to study the effects of HbF-inducing agents on  $\gamma$ -globin chain gene expression (9,10). Xu and Zimmer (11) demonstrated the selective increase of  $G\gamma$  mRNA levels in K562 cells treated with HU. Understanding the mechanisms by which HU differentially regulates HbF gene expression during development may provide data about the mechanisms of action of the pharmacological agents used in SCD.

In the present study, we have determined the effect of HU induction on total Hb level, HbF level and mainly the  $G\gamma/A\gamma$  ratio. In addition, we have compared the relationship between these parameters in order to establish the HbF synthesis patterns in patients with and without HU.

HbF level was determined by the alkaline pH denaturation method (12). In order to separate gel  $G\gamma$  and  $A\gamma$ , HbF was precipitated with sulfosalicylic acid and globin chains were analyzed by polyacrylamide gel electrophoresis, followed by densitometry to determine the proportions of the two chains (13,14). Patients with SCD at Hemocentro/UNICAMP and Boldrini Infantile Center, Campinas, SP, Brazil, treated with HU (group 1, N = 31) were compared with a control group of SCD patients without HU (group 2, N = 30). Control patients were free of crises. All patients but two were older than 13 years. The duration of treatment ranged from 2 months to 5 years (mean time, 2 years).

Group 1 patients had significantly higher levels of Hb, HbF, and  $G\gamma/A\gamma$  ratio than group 2 (Mann-Whitney test; Table 1).

No correlation was found between total

Table 1. Hemoglobin, fetal hemoglobin and  $G\gamma/A\gamma$  ratio in sickle-cell disease (SCD) patients treated with hydroxyurea (HU) (group 1) and in SCD patients not treated with HU (group 2).

Variable	Mean $\pm$ SD	Median	Range
Hemoglobin (g/dl)			
Group 1	9.6 $\pm$ 2.16*	9.5	5.3-16.2
Group 2	8.07 $\pm$ 0.91	8.05	6.6-11.1
Fetal hemoglobin (%)			
Group 1	14.16 $\pm$ 8.31*	12.4	1.0-30.8
Group 2	8.8 $\pm$ 4.09	8.4	2.9-19.8
$G\gamma/A\gamma$			
Group 1	1.45 $\pm$ 0.78*	1.17	0.48-3.25
Group 2	0.98 $\pm$ 0.4	0.87	0.47-1.94

Group 1: N = 31; group 2: N = 30. \*P < 0.05 compared to patients not treated with HU (Mann-Whitney test).

Table 2. Correlation between the variables studied in sickle-cell disease (SCD) patients treated with hydroxyurea (HU) (group 1) and in SCD patients not treated with HU (group 2).

	Group 1	Group 2
Hb and HbF	r: 0.24632 P: 0.1816	r: 0.44291 P: 0.0142
HbF and $G\gamma/A\gamma$	r: -0.17769 P: 0.3389	r: -0.22209 P: 0.2382
Hb and $G\gamma/A\gamma$	r: -0.19738 P: 0.2872	r: -0.37086 P: 0.0436

Hb (g/dl) = hemoglobin; HbF (%) = fetal hemoglobin.

Hb and HbF levels or between Hb levels (total and HbF) and  $G\gamma/A\gamma$  ratio in group 1. However, there was a positive correlation between Hb level and HbF level and a negative correlation between Hb level and  $G\gamma/A\gamma$  ratio in group 2 (Table 2).

Several possible mechanisms have been suggested to explain the action of HU on HbF synthesis. One of them is that HU selectively increases HbF production because, like 5-azacytidine, it may act directly on late erythroid progenitors, reprogramming them to produce more HbF (10,15). An *in vitro* study has shown that the number of burst-forming unit-erythroid colonies decreased whereas HbF levels increased in the presence of HU (16).

The present results, which are in agreement with other studies (2,3,5), demonstrate that treatment with HU increases HbF levels in patients with SCD. In addition, the predominant effect of HU on  $G\gamma$  synthesis was evident since the  $G\gamma/A\gamma$  ratio was significantly higher in the group treated with HU than in the control group. Similar results were reported by Xu and Zimmer (11) using cell culture: the  $G\gamma$  mRNA level increased 2- to 3-fold at 168 h, whereas the  $A\gamma$  mRNA level did not change.

The lack of correlation between HbF levels and  $G\gamma/A\gamma$  ratio suggests that the return to the fetal  $\gamma$  chain synthesis does not imply an absolute increase of HbF production. There is a wide range of factors, both genetic and acquired, that interfere with the increase in HbF production. Among these factors, HbF production can be influenced by cis-acting determinants such as  $\beta$ -globin haplotype and by trans-acting elements such as the X-linked "F cell" production locus. It was demonstrated that the "F cell" production locus is a more important factor in determining HbF levels than  $\beta$ -globin haplotype,  $\alpha$ -globin gene number, or patient age or sex (17). A placebo-controlled study of the efficacy of HU

therapy in sickle-cell anemia patients showed that the Central African Republic (CAR) haplotype was associated with a reduced HbF production response, while patients with the highest baseline granulocyte and reticulocyte counts had the greatest increase in HbF. These data indicate that bone marrow reserve may be an important factor in HbF response. The maintenance of the proliferative capacity of bone marrow in spite of the myelotoxic effects of the drugs is important to sustain HbF increases during HU treatment (18).

In the present study it was not possible to correlate  $\gamma$  chain production and composition with  $\beta$ -globin haplotypes since haplotypes were determined in 26 of 31 treated patients and only in 10 of 30 controls. However, haplotype distribution was similar for the two groups and was divided into Benin and CAR haplotypes according to the distribution reported for the Brazilian Black population (19). A future extensive study may clarify the influence of haplotype on  $\gamma$  chain production stimulated with HU.

The predominant  $\gamma$  chain synthesis induced by HU probably contributes to a milder illness in SCD patients. The contributions of  $A\gamma$  and  $G\gamma$  to the prevention of the sickling process are apparently different. The presence of an alanine ( $A\gamma$ ) instead of a glycine ( $G\gamma$ ) at position 136 of the  $\gamma$  chain interferes with the interaction between  $\gamma$  chain and sickle  $\beta$  chain due to a reduced flexibility of  $A\gamma$ . Thus,  $A\gamma$  is less effective than  $G\gamma$  in preventing the polymerization of the sickle  $\beta$  chain (20).

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