Butyrate-induced reactivation of the fetal globin genes: A molecular treatment for the \(\beta\)-hemoglobinopathies

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Abstract. The inherited \(\beta\)-hemoglobinopathies (sickle cell disease and \(\beta\) thalassemia) are the result of a mutation in the adult (\(\beta\)) globin gene. The fetal globin chain, encoded by the \(\gamma\) globin genes, can substitute for the mutated or defective \(\beta\) globin chain, but expression of the \(\gamma\) globin gene is developmentally inactivated prior to birth. Reinducing expression of the normal fetal globin genes is a preferred method of ameliorating sickle cell disease and the \(\beta\) thalassemias. Stimulation of as little as 4–8% fetal globin synthesis in the bone marrow can produce >20% fetal hemoglobin in the peripheral circulation, due to enhanced survival of red blood cells containing both sickle and fetal hemoglobin, compared to those containing sickle hemoglobin alone. Butyric acid and butyrate derivatives are generally safe compounds which induce fetal hemoglobin production by stimulating the promoter of the fetal globin genes. An initial trial with the parent compound, delivered as Arginine Butyrate, has demonstrated rapid stimulation of fetal globin expression to levels that have been shown to ameliorate these conditions. Phase 1 trials of an oral butyrate derivative with a long plasma half-life have just begun. These agents now provide a specific new approach for ameliorating these classic molecular disorders and merit further investigation in larger patient populations.

Key words. Fetal hemoglobin; sickle cell anemia; \(\beta\) thalassemia; butyrate; gene expression.

The molecular and cellular basis of the \(\beta\) globin disorders

The \(\beta\)-hemoglobinopathies, sickle cell anemia and the \(\beta\) thalassemia syndromes, are among the most common of the genetic diseases, afflicting millions of people worldwide. These disorders were widely selected for because the heterozygous state gave protection against \textit{falciparum} malaria, particularly in early childhood. The tetrameric hemoglobin molecule consists of two \(\alpha\) and two \(\beta\) chains in the adult, enfolding a heme group, to which molecular oxygen binds. The \(\beta\)-thalassemias and hemoglobinopathies are the result of mutations in the \(\beta\) globin gene or a region controlling its expression. In the case of sickle cell anemia, a single base substitution in the globin gene results in the polymerization of hemoglobin upon deoxygenation, with subsequent vaso-occlusion, vascular damage and infarction of internal organs. In the \(\beta\)-thalassemia syndromes, inadequate production of the \(\beta\) globin chain is the result of mutations in the gene or in the gene promotor, resulting in the accumulation of excess \(\alpha\) globin chains, which are toxic to the red cell. The ensuing premature destruction of the red cell produces a severe anemia that usually requires transfusions to sustain life and subsequently the risks of transfusional iron overload\(^{26, 27}\).

The effects of high fetal globin production on the \(\beta\) globin disorders

While individuals with either sickle cell anemia or \(\beta\) thalassemia may be severely affected and often require frequent hospitalizations, it was noted that infants born with these diseases have no symptoms or signs of the disease until after four months of age. It was determined that fetuses and infants are spared the ravages of these diseases during fetal life and into the early perinatal period, the \(\beta^+\) globin gene is not expressed in large quantities and is substituted for by the \(\gamma\) (fetal) globin chain. Although this fetal \(\gamma\) globin chain functions normally in the hemoglobin tetramer, fetal maturation results in an inevitable suppression of \(\gamma\) globin synthesis with a reciprocal increase in \(\beta\) globin synthesis near the end of gestation. This developmental switch occurs even in the setting of defective \(\beta\) globin genes (sickle cell anemia) or the absence of functional \(\beta\) globin genes (\(\beta\) thalassemia).

Clinical studies carried out in Saudi Arabia in the 1970s by Perrine, Woods and Weatherall suggested that continued expression of fetal globin after birth could ameliorate many signs and symptoms of sickle cell disease\(^{26, 27}\). Populations of patients were identified there and in India who were homozygous for the sickle cell mutation. Whereas these patients should have been symptomatic from their disease, especially in their harsh environment, they were found to be in excellent health, to be largely free from sickle crises, and to have normal lifespans. Analysis of globin chain expression in these fortunate patients revealed a persistence of fetal globin expression into adulthood. This genetic abnormality provided sufficient \(\gamma\) globin chains to prevent sickling. Analysis of larger populations revealed that specific levels of fetal
metabolite of ketones, \( \alpha \)-amino-\( n \)-butyric acid, found in
meat, suggesting that an environmental factor present
during their gestation was reversibly inhibiting the
globin expression in the normal term newborn. These
infants of diabetic mothers underwent rapid switching
to predominant \( \beta \) (adult) gene expression when they
were delivered from the diabetic intrauterine environ-
ment. The mechanism by which these agents act is not understood, but it appears likely that some degree of cytotoxic suppression of bone marrow growth is required to produce increases in fetal globin production. Studies using these drugs in adult patients have been underway for a number of years. The ability of these agents to prevent organ damage in sickle cell patients is being investigated in a multi-center trial. In addition, the fact that these drugs are mutagens makes them unattractive as life-long therapies for younger patients with \( \beta \)-hemoglobinopathies. Cytokines which affect erythroid cell progenitor growth have also been utilized to stimulate fetal globin expres-
sion, but these therapies have not resulted in consistent and predictable changes in fetal globin production.

A model of persistent fetal globin expression induced by \( \alpha \)
aminon-\( n \)-butyric acid

In 1985 we conducted a study to determine if the normal developmental fetal globin switch was inhibited in any infant populations. A goal was that study of such a population might lead to the discovery of a natural and safe regulator of fetal globin switching. We found that infants of diabetic mothers did not undergo fetal globin gene switching in utero and were born with a globin expression pattern appropriate for the early fetus of 20–28 weeks’ gestation with 85 to 90% fetal globin synthesis, compared to the pattern of less than 50% fetal globin expression in the normal term newborn. These infants of diabetic mothers underwent rapid switching to predominant \( \beta \) (adult) gene expression when they were delivered from the diabetic intrauterine environment, suggesting that an environmental factor present during their gestation was reversibly inhibiting the switch from \( \gamma \) to \( \beta \) globin chain synthesis. An aminated metabolite of ketones, \( \alpha \)-amino-\( n \)-butyric acid, found in high levels in the plasma of infants of diabetic mothers, was found to be responsible for inhibiting the fetal globin switch in these infants. Despite the inhibition of fetal to adult globin switching, the infants of diabetic mothers in this study were healthy at birth with no signs of other developmental delays. One other development-
gene switch, the alpha fetoprotein to albumin switch, is accelerated in these infants.

Butyric acid, an analogue of \( \alpha \)-amino-\( n \)-butyric acid, had been shown by others to regulate expression of certain genes, including globin, in experimental systems. Sodium butyrate induces expression of a specific embryonic gene globin gene (\( \beta \)) in adult chickens when given in combination with a chemotherapeutic agent. In add-
ition, butyrate had been shown by Partington to regulate the expression of human globin genes after their microinjection into amphibian oocytes. We first tested \( \alpha \)-amino-\( n \)-butyric acid and sodium butyrate for their ability to induce fetal globin gene expression in cultures of erythroid progenitor cells from patients with sickle cell anemia or thalassemia. In culture, exposure to butyrate resulted in significant increases in \( \gamma \) chain synthesis, compared to control untreated culture. In addition, staining for the presence of fetal hemoglobin in young red blood cells, using a specific monoclonal antib-
ody, revealed significant increases in the accumulation of this protein in nearly all the cells in the culture after butyrate exposure.

Studies of butyrate in animal models

In vivo studies with butyrate and butyrate analogs were then begun, using the ovine fetal globin switching model. In these studies, ovine fetuses were infused with butyrate (or saline, as a control) during the later stages of their gestation. In contrast to the human fetal globin switch, which is still underway at term, the ovine switch is complete by the time of birth (day 140). Infusions of butyrate began prior to the start of the ovine fetal switch were capable of preventing the switch. Butyrate-treated fetuses were found to express up to 100% fetal globin synthesis at the time of birth. Although butyrate treatment was quite effective at preventing globin switching if the infusion was begun before globin switching began in this model, the rapid metabolism of this short-chain fatty acid made it difficult to achieve plasma levels sufficient to reverse globin switching once it had begun. A number of analogs of butyric acid were then designed to provide a longer half-life and prevent rapid metabolism. Intratertiary infusion of ovine fetuses with these analogues demonstrated that some of them were not only able to inhibit the fetal globin gene switch prior to its initiation but were also able to reverse the switch once it has occurred. Furthermore, infusion of these analogues could be interrupted for several days, yet high levels of fetal globin expres-
sion were still maintained.