Human Molecular Genetics, 1998, Vol. 7, No. 10 Review 1655–1658

The therapeutic reactivation of fetal haemoglobin

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Received June 6, 1998

Unusually high levels of fetal haemoglobin production can ameliorate sickle cell disease and β thalassaemia. Although efforts directed at the pharmacological stimulation of fetal haemoglobin as an approach to managing these conditions have met with limited success, there is wide variation in individual responses. Whether this reflects the particular mutations that underlie these conditions or other genetic factors remains to be determined, as does the ideal combination of agents to achieve this end. These results are encouraging, however, in particular in view of the recent demonstration that other monogenic diseases, Duchenne muscular dystrophy, for example, might be amenable to the same therapeutic strategy.

INTRODUCTION

It has been realized for a long time that patients with severe inherited disorders of β globin chain structure or synthesis, in particular sickle cell anaemia and β thalassaemia, may have a milder illness if they are fortunate enough to produce unusually high levels of fetal haemoglobin (Hb F) (1). Based on this observation, and the finding that homozygotes for the different forms of hereditary persistence of fetal haemoglobin (HPFH) function normally as adults, with Hb F levels of almost 100%, there has been increasing interest in the possibility of either reactivating or further stimulating Hb F production in these conditions as a novel approach to their management. Although the idea of reactivating genes that are expressed at an earlier stage of development as a way of treating disease has hitherto been restricted to the haemoglobin field, the recent observation that utrophin, the fetal homologue of dystrophin, might be able to replace the action of its adult counterpart in mice with Duchenne muscular dystrophy (2) suggests that this approach might have more general application for the management of monogenic or other diseases.

Partly because of lack of genuine progress towards an understanding of the mechanisms that regulate the switch from embryonic to fetal and fetal to adult haemoglobin during normal development, progress in finding ways to encourage persistent fetal haemoglobin production, or to reactivate it, has been slow. However, there have been some limited successes. Because the haemoglobin disorders are becoming increasingly common, their symptomatic treatment with blood transfusion and iron chelation poses many problems, bone marrow transplantation is not always possible, and there is little sign of any immediate progress on the gene therapy front, it is important that the lessons learnt so far are fully understood so that further studies can be designed to try to obtain more consistent results.

FETAL HAEMOGLOBIN PRODUCTION DURING NORMAL DEVELOPMENT

At each stage of development, different haemoglobins are produced, each of which consists of two unlike pairs of globin chains (3). In the embryo, ζ chains combine with ε or γ chains to produce Hbs Gower 1 ($\zeta_2\varepsilon_2$) and Portland ($\zeta_2\gamma_2$), and α chains combine with ε chains to form Hb Gower 2 ($\alpha_2\varepsilon_2$). After about the 12th week of gestation, α chains combine with γ chains to produce Hb F ($\alpha_2\gamma_2$) and, during the third trimester, γ chain synthesis starts to decline and is replaced by β and δ chain production, with the formation of the adult haemoglobins A ($\alpha_2\beta_2$) and A_2 ($\alpha_2\delta_2$). Fetal haemoglobin consists of two molecular forms, $\alpha_2\gamma_2^{136Gly}$ and $\alpha_2\gamma_2^{136Ala}$; the $^{G}\gamma$ and $^{A}\gamma$ chains are the products of linked γ chain loci. The α-like genes form a cluster on chromosome 16 in the order ζ–α2–α1, and the β-like genes are on chromosome 11, in the order ε–$^{G}\gamma$–$^{A}\gamma$–δ–β. Thus, both clusters, which also contain several pseudogenes, carry the globin genes in the order in which they are expressed during development, in a 5′→3′ orientation.

Fetal haemoglobin replaces adult haemoglobin by the end of the first year of life. All normal adults produce a small quantity, ~1%, which appears to be confined to a small population of red cells which are called F cells (3). Studies of clonal haemopoietic malignancies have shown that the F cell population is not the progeny of a particular haemopoietic stem cell line. Whether there are small amounts of fetal haemoglobin in most adult red cells, and F cells simply represent the end of the spectrum at which it is detectable, is uncertain. However, there is a strong genetic component to the relative number of F cells; at least one locus, on the X chromosome, has been implicated, although its identity and mode of action remain to be determined (4).

Very little is known about the regulation of the switch from fetal to adult haemoglobin (3,5), only one of several well defined developmental changes of the red cell proteins. Both sequential

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studies of normal infants and transplantation experiments in sheep suggest that the time of switching is related to gestational age and not to the time of birth. Current models suggest that the locus control region (LCR), a major regulatory sequence 5′ to the ε globin genes, becomes opposed sequentially to the ε, γ, δ and β loci at particular times during development (5). Whether this reflects the interaction of developmental stage-specific regulatory proteins remains to be determined. It is of interest that during normal development, in conditions in which there is persistent fetal haemoglobin production, and in transgenic mouse models, there appears to be a reciprocal relationship between γ and β and δ chain production, suggesting that there may be competition for the factors which are involved in interactions between the LCR and the globin gene promoters.

PERSISTENCE OF FETAL HAEMOGLOBIN PRODUCTION

Persistent production of variable levels of Hb F into childhood and adult life is a characteristic finding in sickle cell anaemia and the more severe forms of β thalassaemia. However, because relatively high levels of Hb F in red cells protect them against sickling, and because γ chains combine with some of the excess α chains that result from defective β chain production in β thalassaemia to produce Hb F, the relatively high levels of Hb F in the peripheral blood in these conditions probably reflects F cell selection in the marrow and blood, possibly combined with marked erythroid hyperplasia in response to anaemia (6). This interpretation is strengthened by recent studies which have shown that if the bone marrow is suppressed in β thalassaemics by regular transfusion the baseline level of Hb F production is a little greater than normal (7). Thus, although there may be some fine tuning of the level of Hb F production in β thalassaemia as a result of the underlying mutations, those which involve the promoter regions of the β globin genes being associated with higher levels, the major mechanisms involved in its postnatal synthesis appear to be cell selection, probably combined with marked erythroid expansion, which seems to favour γ globin gene reactivation.

There is a family of conditions that comprise HPFH, in which there is persistent fetal haemoglobin production in the absence of haematological abnormalities. The underlying mutations consist of either long deletions which remove the δ and β globin genes, or point mutations in the region of the γ gene promoters (3,5). In addition, there are varieties which are determined by loci that are on other chromosomes, notably chromosome 6 (8). The way in which these lesions produce persistent fetal haemoglobin production is not clear. In the case of the promoter mutations, this could reflect a γ gene promoter of increased efficiency, or an inability to bind repressors of Hb F production in adult life. However, whatever the mechanism, the very high levels of Hb F that are produced in HPFH indicate that adult red cell precursors contain all the factors required for fetal haemoglobin synthesis.

REACTIVATION OR MODIFICATION OF THE LEVEL OF FETAL HAEMOGLOBIN

Early observations of transient increases in fetal haemoglobin production in patients recovering after treatment with cytotoxic agents for leukaemia (9), following marrow transplantation (10), or in animals after phlebotomy (11), suggested that acute erythroid expansion might be associated with fetal haemoglobin synthesis. It was reasoned that this phenomenon, possibly associated with perturbations of the cell kinetics of erythroid progenitors as a result of exposure to anti-leukaemic agents such as hydroxyurea and cytosine arabinoside, might lead to a premature commitment to an erythroid maturation pathway with an increased likelihood of fetal haemoglobin production (12). The observation that babies born of diabetic mothers have higher fetal haemoglobin levels than normal, and that the switch from fetal to adult haemoglobin production might be delayed (13,14), together with *in vitro* experiments suggesting that this effect may be mediated by butyrate (15), suggested that, in addition to cell cycle-specific agents, butyrate compounds might also be worth pursuing as potential fetal haemoglobin switching agents.

THE RESULTS OF CLINICAL TRIALS OF AGENTS DESIGNED TO REACTIVATE OR AUGMENT FETAL HAEMOGLOBIN PRODUCTION

Based on these observations, a number of clinical trials directed at augmenting Hb F production in sickle cell anaemia or thalassaemia have been carried out using different agents, either alone or in combination (16).

Based on preliminary studies of the effects of the S-phase-specific agent hydroxyurea in patients with sickle cell disease (17–19) on Hb F production, several clinical trials were carried out to establish its efficacy. In a large well designed placebo-controlled trial in adults, it was found that the number of painful crises, episodes of the chest syndrome and transfusion in sickle cell disease were reduced and that there was an approximate doubling of the mean level of Hb F and F cells; the Hb F rose from a baseline of ~5–9% (20). While individual patients showed a much greater rise in Hb F, this modest overall increase is unlikely to have been sufficient to account for the clinical benefits. Hydroxyurea has a variety of other effects, including increasing the red cell size and, therefore, presumably altering cellular hydration, and reducing the white cell count. Both these factors may have played a role in reducing the number of painful crises. However, this important study showed quite unequivocally that Hb F can be elevated safely by the use of hydroxyurea, and more recent trials have shown that the same effect can be obtained in children (21–23). It should be emphasized that the long-term safety of this form of therapy remains to be determined.

In studies in which hydroxyurea has been administered to patients with different forms of β thalassaemia, there have been only minimal responses in terms of an increase in haemoglobin or Hb F levels (24–30). A small number of patients with β thalassaemia treated with 5-azacytidine showed an increase in γ globin mRNA synthesis, normalization of globin chain imbalance and an increase in the total haemoglobin concentration, exceeding 2 g/dl (31,32). This drug is, however, potentially toxic and hence has not been studied further. Cytosine arabinoside has been explored in animal and human studies; the numbers treated are too small to reach any definite conclusions about its efficacy.

Because of reports of suboptimal erythropoietin (Epo) response to anaemia in both sickle cell anaemia and β thalassaemia, and indirect evidence that rapid erythroid expansion may favour Hb F production, there have been several studies to assess the effects of recombinant Epo in these disorders, either alone or in combination with hydroxyurea (33–39). Although the results have been variable, there are potential risks in raising the haemoglobin level without a major increase in Hb F in patients

with sickle cell anaemia, and erythroid expansion itself may cause increased iron absorption and extramedullary haematopoiesis; these studies suggest that it will be well worthwhile further exploring the effects of combinations of Epo with other agents.

The administration of butyrate compounds has also been associated with varying responses. In an initial study, involving the intravenous administration of arginine butyrate, one patient, a homozygote for haemoglobin Lepore, showed a dramatic response with a major rise in both haemoglobin and Hb F values (40). However, in a subsequent study of the use of this agent in 10 patients with different types of β thalassaemia, only modest or no changes in haemoglobin or Hb F levels were noted (41). Because of difficulties in long-term treatment with arginine butyrate, the original patient with haemoglobin Lepore was treated over a long period with a combination of sodium phenylbutyrate and, later, hydroxyurea. Overall, this patient had a dramatic response to this combination of drugs, with a total rise of haemoglobin to 10–11 g/dl, reflecting an increase of Hb F of ~5 g/dl. She has remained non-transfusion-dependent and well for >4 years (42). For this reason, her transfusion-dependent brother was treated with a combination of sodium phenylbutyrate and hydroxyurea and had an almost identical response; he has been independent of transfusion for nearly 2 years (42). One other patient, in this case a compound heterozygote for haemoglobin Lepore and β thalassaemia, showed a good response to hydroxyurea alone, with a rise in the haemoglobin of 4 g/dl (43). In a pilot study of 11 adults with β thalassaemia treated with oral sodium phenylbutyrate, four showed a rise in total haemoglobin of ~1 g/dl (44); the same agent also produced a modest rise in Hb F in some patients with sickle cell anaemia (45). There have been some reports of the efficacy of pulsed doses of sodium phenylbutyrate in patients with sickle cell anaemia and thalassaemia, although full details have not yet been published (46).

The finding that in the majority of patients with different forms of β thalassaemia studied to date there is no Hb F response, or only a modest rise, whereas a few, when given the same agents, have a quite dramatic rise in Hb F and total haemoglobin levels, even to the extent of allowing them to remain independent of blood transfusions, raises some important issues. The siblings homozygous for haemoglobin Lepore were studied in considerable detail during their remarkable response to butyrate and hydroxyurea (42). There was contraction of their erythroid mass, indicating that the unusual rise in Hb F is not mediated by stimulation of erythroid expansion. As judged by the $^{G}\gamma/^{A}\gamma$ composition of their Hb F and the patterns of their other red cell proteins, they showed no evidence of a reversion to fetal erythropoiesis and no other fetal-stage-specific proteins were detected in their blood. Furthermore, detailed family studies did not disclose any other genetic determinant which might be contributing to this unusual response. These observations raise the important question as to whether responses to these pharmaceutical agents depend, at least to some degree, on the underlying molecular lesions that cause β thalassaemia. Haemoglobin Lepore is an uncommon form of the disease in which there is an abnormal crossover between the linked δ and β genes with the production of δβ and βδ fusion loci; the product of the δβ fusion locus is synthesized at a low rate and is associated with the phenotype of β thalassaemia. As part of the abnormal crossing over, the 3′ end of the δ and the 5′ end of the β genes are lost together with the region between them. It is possible that this short deletion is in some way responsible for the unusual response to hydroxyurea and butyrate.

CONCLUSIONS

The results of the hydroxyurea trials for sickle cell anaemia are very encouraging, although the augmentation of fetal haemoglobin is unlikely to have been sufficient to explain all the beneficial effects of this agent. Overall, the outcome of the limited trials to treat thalassaemia have been less encouraging. However, there are some remarkable exceptions and it is now very important to determine whether these unusual responses reflect the action of the particular underlying thalassaemia mutations or whether other mechanisms are involved. These questions can be answered by appropriate clinical trials and studies of patients in which the action of these pharmacological agents is related specifically to their underlying molecular defects as well as to other factors that might alter individual responses to switching agents, both genetic and acquired. Another problem which requires further exploration is whether particular combinations of these agents may be more effective than single agents, and, if so, precisely how they should be administered and at what dosage.

Given the wide range of factors, both genetic and acquired, that set the level of Hb F in patients with β globin disorders, we should not be surprised to find that there is wide variability in the response to these non-specific approaches to the augmentation of fetal haemoglobin synthesis. While further studies may yield more definitive ways of augmenting Hb F production, based on developmental stage DNA-binding proteins for example, these advances may be a while in coming. In the meantime, we must learn to use the pharmacological agents that we have at our disposal more effectively. Our objectives can be modest; a rise in steady-state haemoglobin level of 2–3 g/dl in patients with intermediate forms of β thalassaemia, Hb E thalassaemia for example, would be extremely beneficial.

Although these early results from the globin field have shown that it is possible to reactivate or augment the production of a fetal protein to at least partly replace its defective adult counterpart, the approaches that are being explored, because of their lack of specificity and, at least in some cases, dependence on perturbations of the rapidly turning over erythroid population of the bone marrow, may not have direct relevance to other genetic diseases. Even the long-term administration of butyrate compounds, although clearly up-regulating the γ globin genes, shows no evidence of reactivating of other fetal genes (42). Thus the long-term goal must be to define the developmental stage-specific *trans*-activating regulatory proteins involved, with a view to evolving more specific ways to ‘switch on’ fetal genes during later development.

ACKNOWLEDGEMENTS

This work was supported in part by the Medical Research Council and the Heart and Stroke Foundation of Canada, and the Medical Research Council and Wellcome Trust of the UK. N.F.O. is a Scientist of the Medical Research Council of Canada.

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