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SICKLE CELL DISEASE: NEW TREATMENT INSIGHTS

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SUMMARY

The clinical description of sickle cell disease (SCD) occurred over a century ago and yet cure of this relatively common genetic disorder remains limited to the few patients undergoing HLA-matched allogeneic hematopoietic stem cell transplantation (HSCT). Many pharmacotherapeutic approaches have been attempted and hydroxyurea has recently become more widely accepted as an effective agent. Standard practice for stroke prevention is chronic red cell transfusion, but cumulative iron overload can be prohibitive. Gene therapy for SCD offers a potential new curative option that aims to avoid the treatment-related toxicities of allogeneic HSCT and offer cure to more eligible patients than HSCT. Clinical gene therapy trials for hemoglobinopathies are under way in Europe and soon to follow in the U.S. Finally, great potential has been unlocked in the recently described induced pluripotent stem (iPS) cell derived from somatic human tissue. While the science of iPS cells is still in its infancy, these cells may provide a platform in the future to correct the sickle mutation and derive healthy long-term HSCs for clinical use.

BACKGROUND

It has been about 100 years since Dr. James Herrick commented on a patient with "sickle-shaped" and "crescent-shaped" red blood cells on the peripheral blood film, the first clinical description in Western medicine of sickle cell disease (SCD) (1). It was, however, the work of Linus Pauling in 1949 that sparked interest in a molecular basis for the disease, and in 1956 Ingram and Hunt demonstrated that the hydrophilic glutamic acid at position 6 of the β-globin chain was replaced by the hydrophobic valine. The disease is now known to be caused by a single point mutation leading to an amino acid substitution that results in a phenotype of significant disease morbidity and shortened life span. Progress in treatment has been made with hydroxyurea, which has gained favor in both pediatric and adult hematology clinics. While hematopoietic stem cell transplantation (HSCT) is currently the only curative approach, gene therapy appears to be moving ever closer to providing an eventual cure. In the last few decades, increased insight into the pathophysiological mechanisms of the disease has led to the development of new and different therapies for SCD. Here, the current practice treatments and promising therapies on the horizon are reviewed.

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TRANSFUSION THERAPY AND MANAGEMENT OF IRON OVERLOAD

In 1969, Lusher and colleagues described a prophylactic transfusion program for a group of pediatric sickle cell patients with central nervous system (CNS) infarction, where the goal was to reduce the total amount of sickle hemoglobin (HbS) in the blood by reducing the percentage of endogenous red cells that contained HbS. A transfusion interval of 3 weeks was empirically chosen and the goal was to achieve a level of normal adult hemoglobin (HbA) of at least 60% prior to each transfusion. They noted that low reticulocyte counts correlated with little or no detectable HbS. They would later add pre-transfusion phlebotomy to their regimen to minimize iron overload caused by the prophylactic transfusions. This practice was sufficient to prevent the progression of CNS symptoms and allowed two-thirds of the patients to improve (2).

The STOP trial published in 1998 definitively showed that chronic transfusion therapy is effective at reducing the risk of first stroke in pediatric patients with abnormal transcranial Doppler (TCD) velocities by about 90% when a goal of 30% HbS pre-transfusion is targeted (3). This 30% goal has become the standard of care. The STOP 2 trial published in 2005 went on to show that stopping transfusions aimed at preventing primary stroke resulted in a high rate of stroke and a return to abnormal TCD velocities (4). Based on these findings,

patients with abnormal TCD velocities benefit from stroke prevention with transfusion therapy, but unfortunately, take on the inherent risks of iron overload, blood-borne pathogen transmission and alloimmunization to red cell antigens. Attempts to limit iron overload include phlebotomy and red cell exchange/erythrocytopheresis. Iron chelation therapy has been used in conjunction with chronic transfusion for quite some time, with desferrioxamine typically given by s.c. infusion over 8-12 h nightly. A less cumbersome approach now used is daily administration of the oral chelator deferasirox. However, despite some reports suggesting equal efficacy to desferrioxamine in this patient population (5), adherence can be quite variable and seemingly suboptimal, especially in the adolescent age group (5, 6).

Ultimately, iron overload remains an ongoing concern for patients receiving chronic transfusion therapy and it appears that other treatment approaches for stroke prevention need to be developed and tested.

PHARMACOLOGICAL THERAPY TO INDUCE FETAL HEMOGLOBIN

As is the basis for many medical approaches, nature has provided a model for the treatment of SCD in the form of increased expression of fetal hemoglobin (HbF). It has been observed that patients who have a hereditary persistence of HbF can have up to 10-40% HbF contributing to the total hemoglobin (7). Patients who have SCD and such a persistent elevation of HbF can have a milder course of disease that is related to the level of HbF expression (8).

Hydroxyurea

Hydroxyurea, or hydroxycarbamide, is an agent used to increase HbF expression in the red blood cells of patients with SCD. The understanding of the mechanism of this increase remains incomplete. A major element of its effect appears to reside in its ability to inhibit ribonucleotide reductase, thereby inhibiting DNA synthesis and increasing cytotoxicity in late red cell progenitors (9). Perhaps this action alters the kinetics of erythropoiesis, with the release of late erythroblasts and reticulocytes containing higher levels of HbF (10). Changes are also likely to occur at the level of the chromatin in the β-globin locus. Specifically, changes could occur in the chromatin of the γ- and β-globin promoters that might alter the ratio of γ to β expression, leading to greater amounts of HbF ($\alpha_2\gamma_2$) and less HbA ($\alpha_2\beta_2$).

Hydroxyurea has been shown to reduce the incidence of painful crises and acute chest syndrome (11). Evidence is building that shows protection against chronic end organ injury (12, 13). With respect to CNS disease, the combination of hydroxyurea administration coupled with phlebotomy is being tested as an alternative to chronic transfusion in secondary stroke prevention in the "Stroke With Transfusions Changing to Hydroxyurea (SWiTCH)" trial (14). Recently, this trial was halted due a lack of difference being observed in the iron overload parameters measured between the two groups.

In the last few decades, hydroxyurea has been shown to be a generally well tolerated and safe treatment for SCD in adults (11, 15). Hydroxyurea has also become more attractive for the treatment of pediatric patients, given improvements seen when the drug is used at the maximum tolerated dose (16, 17). To date there have been no significant long-term side effects noted with the clinical use of hydroxyurea in children. The ongoing trial titled "Long Term Effects of Hydroxyurea Therapy in Children With Sickle Cell Disease" aims to address the long-term cellular, molecular and clinical effects of hydroxyurea (18).

Decitabine

Decitabine is a pharmacological agent that has been evaluated for patients with SCD given its ability to increase HbF. Decitabine is an analogue of cytidine that when incorporated into DNA can inhibit methylation enzymes and reactivate transcription. The effect of decitabine on DNA hypomethylation may be linked to the increase in HbF expression (19, 20). Currently, the drug is available for s.c. injection and the development of an oral form is under way. Clinically significant increases in HbF and total hemoglobin have been seen in phase I/II trials of decitabine in sickle cell patients (19, 21, 22). However, concerns over carcinogenic activity of the closely related 5-azacytidine in preclinical studies have slowed efforts to conduct a phase III trial (23-25). Furthermore, there are valid theoretical concerns over teratogenicity and negative effects on the male reproductive system (25). Nevertheless, as decitabine has been used effectively in patients with significant manifestations of SCD who were deemed to have not responded to hydroxyurea, further investigation of this drug seems warranted (25, 26).

Butyrate

The delay in switching from γ-globin expression to β-globin in infants of diabetic mothers was demonstrated in the 1980s to be associated with the elevated amounts of butyrate found in their plasma (27-29). Butyrate is a histone deacetylase inhibitor and it is proposed to increase HbF expression by a resulting increase in production and translation of γ-globin mRNA (30). Clinical trials using continuous infusions of arginine butyrate in SCD patients have resulted in mixed responses, with some patients responding and others not (31, 32). However, intermittent infusions may yield more sustained increases in HbF levels (33). Oral sodium phenylbutyrate may increase HbF expression in adults, but the dosage required necessitates ingesting up to 40 tablets daily and adherence would be a concern (34). In children, a pilot study of low-dose continuous oral sodium phenylbutyrate increased the percentage of F reticulocytes and HbF, but ultimately the effect diminished (35). Clinical use of butyrate is limited, as the most effective trials have used i.v. infusions. Work towards an intermittent oral formulation may be beneficial, especially in combination therapy, as butyrate lacks the myelosuppressive toxicity seen with other therapies.

RED CELL DENSITY

The polymerization of sickle red cells is potentiated by increased concentrations of HbS (36) and SCD patients are advised to avoid dehydration. Potassium loss and subsequent dehydration that occur in sickle cells are mediated by potassium/chloride cotransporters and calcium-dependent potassium channels (Gardos channels) on the cell surface (37). Thus, efforts have focused on targeting these to reduce red cell density.

Senicapoc

Senicapoc (ICA-17043) is an agent that selectively and potently inhibits Gardos channel activity and thus prevents the movement of potassium out of sickle cells through these channels. In a phase II clinical trial of senicapoc for SCD, the hemoglobin level increased and markers of hemolysis improved (38). A phase III trial of senicapoc in SCD to reduce painful crises was stopped, as it became clear that there was a low probability for a decrease in this primary outcome of painful crises (39). Further evaluation for a senicapoc is needed to determine if the reduction of hemolysis may have an impact on chronic organ damage.

Magnesium

Increased magnesium levels in sickle red cells can stop the efflux of potassium ions and prevent dehydration when compared to cells with depleted magnesium (40). This occurs through the ability of magnesium to inhibit the potassium/chloride cotransporters (40, 41). There have been limited clinical trials to date studying the potential efficacy of magnesium. In one trial, oral magnesium pidolate given to 10 patients resulted in increases in red cell magnesium and potassium levels and a decreased number of dense sickle cells (42). Another trial showed decreased painful events after starting magnesium supplementation in 20 SCD patients, but interpretation of these results was limited by the trial being open-label and unblinded (43).

There have been efforts to use magnesium in combination with hydroxyurea. A phase I trial in pediatric SCD patients taking hydroxyurea in combination with magnesium pidolate (pyroglutamate) suggested some benefit in laboratory parameters, but this was restricted to those with the worst red blood cell abnormalities (44). Results are pending for the recently concluded "Hydroxyurea and Magnesium Pidolate to Treat People With Hemoglobin Sickle Cell Disease (CHAMPS)" trial (45). Ultimately, the clinical efficacy of magnesium as a potentially inexpensive and well-tolerated therapy for SCD remains to be proven.

Erythropoietin

With many red blood cells bearing deoxygenated, polymerized HbS and microvascular occlusion being chronic and ongoing, tissue hypoxia is common in virtually all tissues of patients with SCD. As a result, the already activated hypoxemic-driven erythropoietin response in SCD patients may limit potential therapeutic benefit by the provision of additional exogenous erythropoietin. However, in the setting of renal dysfunction, as is often the case in older adults with SCD, the erythropoietin response can be blunted (46). Thus, the concept of combination hydroxyurea and erythropoietin may have a role in certain patient populations. Erythropoietin in combination with hydroxyurea and stem cell factor was shown to have additive effects on HbF expression in the baboon (47). Small studies in patients with SCD have shown mixed results with the erythropoietin/hydroxyurea combination (48-50). A single-center experience, however, showed efficacy for the combination treatment in SCD patients with severe anemia, reticulocytopenia and renal dysfunction (46), and suggested that this may be a potential niche for this therapy.

HEMATOPOIETIC STEM CELL TRANSPLANTATION

Hematopoietic stem cell transplantation (HSCT) remains the only curative therapy currently available for SCD. The first successful bone marrow transplant was described in 1984 and involved a patient who was transplanted for acute myelogenous leukemia but who also had SCD. The patient was cured of both diseases (51). It remains a point of discussion, and in some circles controversial, as to the criteria which should be used to decide on transplant candidates. The first large series of HSCT for SCD, published in 1996, used inclusion criteria that are still applied today. The criteria were that patients be less than 16 years old, an HLA-identical related donor be available, and the patient have had at least one of the following: stroke or CNS event lasting more than 24 h, acute chest syndrome, recurrent severe pain episodes, impaired neuropsychological function and abnormal magnetic resonance imaging of the brain, stage I or II sickle lung disease, sickle nephropathy, bilateral proliferative retinopathy, osteonecrosis of multiple joints, or red blood cell alloimmunization during long-term transfusion therapy (52).

There have not been any randomized, controlled trials published evaluating different types of HSCT or comparing HSCT to other preventative or supportive measures (53). In the last two decades significant data have been collected showing event-free survival of 70-80% and overall survival of > 90% in patients treated with HSCT for SCD (52, 54-57). It should be noted that these HSCT utilized HLA-matched related donors, which drastically decreases the number of patients eligible to receive a transplant. This problem was demonstrated in a survey of participants in a multicenter study, where of 4,848 patients with SCD, 315 met inclusion criteria for transplant, 128 went on to have HLA typing done and only 44 (0.9%) had an HLA-identical sibling (58). This has prompted work on alternative donor graft transplants, including the consideration to utilize unrelated cord blood and bone marrow. The latter are being evaluated in the multicenter study "Unrelated Donor Hematopoietic Cell Transplantation for Children with Severe Sickle Cell Disease Using a Reduced Intensity Conditioning Regimen, The SCURT Study" (59).

Successful HSCT for SCD has employed a myeloablative conditioning regimen aimed to achieve high engraftment rates (52, 54-57). The myeloablative approach has a significant associated risk of toxicity and may exclude patients from treatment, especially given the chronic multiorgan injury that is characteristic of SCD. HSCT is becoming more available to patients with malignant hematological disease who may not tolerate the toxicity of myeloablative regimens with the use of so-called reduced-intensity conditioning regimens (60-62). Such a novel approach was used by Tisdale and colleagues in a study of 10 adult patients with SCD. The patients received HLA-matched, related HSCT after a submyeloablative preparative regimen (consisting of low-dose total body irradiation, alemtuzumab (an antibody to CD52) and sirolimus as an immune modulator with tolerogenic activity. The authors reported remarkable efficacy with limited toxicity (63).

To date, allograft sources remain limited to related donors for the best results. Alternative approaches to curative cellular therapy are thus being pursued. Gene modification therapy applied to autologous hematopoietic stem cells (HSCs) offers the advantages of the

potential for less intensive cytoreductive conditioning regimens and will avoid the immunological risks associated with allogeneic transplant, namely graft versus host disease and infection.

GENE THERAPY

The first publication of gene therapy showing hematological correction and decreased organ damage in a mouse model of SCD appeared in 2001 (64). Gene modification was effected by a lentiviral vector based on HIV that lacks the ability to replicate and inactivates once genomic integration has taken place. Lentiviral systems have improved safety profiles as compared to oncoretroviral vectors, which have been associated with leukemic transformation, as was seen in X-linked severe combined immunodeficiency clinical trials (65-67). The vector used to correct the murine SCD model introduced an anti-sickling β-globin engineered to contain the believed anti-sickling portion of naturally occurring γ-globin (64). Another approach utilized vectors containing γ-globin itself, as HbF is known to naturally decrease the polymerization of deoxygenated HbS (68). It is proposed that, as HbF occurs naturally at varying levels in SCD, there is less concern for an immune response compared to a potential neoantigen that could be derived from the mutated, anti-sickling β-globin protein (69). In the mouse model, this γ-globin approach can also improve or cure the SCD hematological abnormalities, as well as ameliorate the characteristic organ pathology of SCD (69). In this study, a significant finding was that phenotypic improvement occurred at a relatively low average vector copy number per cell in the bone marrow (0.3-1.8 copies). This is an important characteristic, since increased numbers of vector integrations per cell are undesirable, as they may increase the risk of aberrant cell function or proliferation.

A clinical gene therapy trial for β-thalassemia and SCD is ongoing in France (70). A vector containing the anti-sickling β-globin shown to correct SCD in a mouse model is being used and can be distinguished from low-level expression of endogenous β-globin in β-thalassemia. To date, two patients have been treated by infusion of their own $CD34^+$-enriched bone marrow after it was transduced with the globin lentiviral vector (71). The first patient, with transfusion-dependent β-thalassemia major, was conditioned with myeloablative doses of busulfan, and despite infusion of the transduced $CD34^+$, engraftment was prolonged and the persistent pancytopenia of the patient required infusion of frozen "backup" untransduced autologous cells. The second patient, with hemoglobin E (HbE)/β-thalassemia, also underwent infusion of gene-modified cells after busulfan conditioning. Hematological engraftment was evident by 5 weeks and the patient, previously transfusion-dependent, went on to become transfusion-independent. Following gene therapy, the patient was observed to express equal amounts of HbE, the anti-sickling β-globin and HbF. The HbF may be an effect that occurred after myeloablation and contributes in part to the clinical improvement in the patient; however, HbF is often elevated in the context of HbE/β-thalassemia, so it may be that this is part of the pre-existing clinical condition. About 2 years after gene therapy, it was reported that of the 10% of gene-modified cells there was a relative clonal dominance of cells (about half of the 10%) that contained an integration in the *HMGA2* gene, a potential oncogene (72, 73). The patient, up until the latest reports, has remained well and the clonal population has been stable. It is possible that the clonal observation represents engraftment with only a small number of transduced hematopoietic stem cells following transplant. Thereafter, the cells may have normal function, but their red cell progeny have a selective advantage given the decreased survival of the naturally occurring β-thalassemia erythrocytes. Clinical trials for β-thalassemia and SCD are planned in the U.S., including at our own institution (74).

INDUCED PLURIPOTENT STEM CELLS

In 2006, the generation of induced pluripotent stem (iPS) cells from mouse fibroblasts using the transcription factors Oct-4, SOX-2, Krueppel-like factor 4 and c-Myc was reported (75). The significant implication of these findings was the ability to produce cells of equal potential to an embryonic stem cell from a somatic cell source (fibroblast, blood cell, etc.). This would provide a platform for studying the differentiative process of a large number of cell types which can be derived from iPS cells. In 2007, the same group went on to show that iPS cells could be made from human adult fibroblasts (76). The method originally employed to produce iPS cells used a viral vector system, which raised concerns about mutagenic insertions. Recently, it has been shown that iPS cells can be generated without viral vectors, but rather by transient transfection with non-integrating gene delivery systems (77, 78). Application of the recombinant proteins that mediate reprogramming has also been accomplished to generate iPS cells (79). These efforts to create a process devoid of oncogenes that still results in an efficient reprogramming and production of quality iPS cells will be important (80).

While the clinical application opportunities of iPS cells seem infinite, many aspects need to be addressed, including a feasible method to collect sufficient somatic cells from a patient, efficiency of the reprogramming process for clinical scale, rigorous safety evaluations of the iPS cells and the longevity of the tissue derived from iPS cells (81). Preclinical proof-of-principle studies have shown correction of disease in SCD using hematopoietic progenitors derived from gene-modified iPS cells (82). This work is very supportive of a potential cure for SCD using iPS cells, although there are some specific areas that will need to be addressed before advancing to clinical trials. Ideally, and unlike in the proof-of-principle experiments, the iPS cells should be derived without the use of viral vector gene modification and the hematopoietic cells derived without co-culturing systems (81). Finally, proof that a long-term, true repopulating hematopoietic stem cell can be derived from iPS cells still remains to be shown. This therapy, while it has a very promising future, may be realistically decades away from clinical practice.

CONCLUSIONS

Great strides have been made in the primary medical treatment of SCD in the last 100 years. These advances are due in no small part to understanding the molecular basis of the disease and the mechanisms of HbS polymerization and sickling erythrocytes. Much has been learned about the chronic end organ damage that is essentially universal in the disease. Attempts to increase HbF pharmacologically have led to the utilization of drugs such as hydroxyurea and decitabine. Hydroxyurea is gaining more acceptance in its preventive role and greater efficacy is seen when the drug is taken at the maxi-

mum tolerated dose. Decitabine is currently being used as a treatment for disease refractory to hydroxyurea therapy, although further investigation may broaden its application. Combination therapies such as hydroxyurea and magnesium pidolate are currently being investigated in pilot studies, as attempts are made to boost the correction of sickle pathology by attacking different disease mechanisms without additive toxicity. The hematopoietic transplantation field continues to improve, particularly with significant recent advances reported for less toxic conditioning regimens and identifying new graft sources. This is especially important, since primary and supportive care for SCD has significantly improved and transplantation should not expose patients to an undue higher risk of mortality. Gene therapy is very promising and the ongoing trials using gene vector transfer systems for hemoglobinopathies are pivotal. While the source of HSCs available for gene modification in SCD is currently limited to steady-state bone marrow, iPS cell technology opens a whole new world to apply the techniques of gene therapy and may provide the best and most widely available chance for cure.

DISCLOSURES

The authors state no conflicts of interest.

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