PHARMACOLOGIC THERAPIES FOR SICKLE CELL DISEASE

Special Edition with Guest Editor
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Editor-in-Chief

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The Foundation for Sickle Cell Disease Research (FSCDR) established The Journal of Sickle Cell Disease and Hemoglobinopathies (JSCDH), a peer-review journal. It caters to physicians, scientists, allied healthcare, public health professionals, pharmaceutical and biotechnology researchers and developers, patients and caregivers engaged in Sickle Cell Disease and hemoglobinopathies.

The goal of the JSCDH is to have a 360-degree view of Sickle Cell Disease and Hemoglobinopathy. This creates an environment for rapid advancements of drug and therapeutic development, improvement of clinical infrastructure and identification of pressing public health issues.

Understanding the mechanisms of disease through basic science research engages with those living with sickle cell disease and supports caregivers. The JSCDH publishes select, state-of-the-art reviews, clinical studies, experimental investigations, new therapeutics, case reports, editorials and articles.

We are in the midst of a non-theoretical paradigm for Sickle Cell Disease. The sickle gene is significantly embedded in the framework of human development. It continues to elude the most rigorously designed scientific, clinical and public health models and their ability to purge the toxic effects of a double dose of the protective single hemoglobin S gene and its variants.

The sickle gene’s protective nature is always active, just as effortlessly as it is for us to walk, breath and blink. The intention is to protect its host from parasitic malaria takeover. This harmonized resistance occurs in the most populous areas of the world, speaking to the importance of the sickle gene for worldwide survival.

We’re on a journey to make an evolutionary perfected process imperfect, for the sake of relieving pain and suffering. The battle against hemoglobin S is to conquer its mastery of polymerization and oxygen deprivation. Fittingly, those affected by sickle cell referring to themselves as Warriors.

Our attempt to alter the evolutionary history of hemoglobin S is reflected in the first full manuscript of the JSCDH. This includes an assessment of new pharmacologic therapeutics by guest editor, Kenneth I. Ataga in Section I, and insights into the worldwide clinical and social consequences of sickle cell disease in Section II.

I am grateful to my colleagues who contributed to this first published full manuscript. Your contribution to launch the first journal dedicated to sickle cell disease research will earmark an evolution of global partnership to accomplish transformative research.

Lanetta Bronté , MD, MPH, MSPH
Editor-in-Chief
Journal of Sickle Cell Disease and Hemoglobinopathies
Biography: Dr. Kenneth Ataga is an adult hematologist with a clinical and research interest in sickle cell disease. He attended medical school at the University of Benin, Benin City, Nigeria, following by a residency in Internal Medicine at Upstate Medical University (formerly State University of New York Health Sciences Center) at Syracuse, NY and a fellowship in Hematology-Oncology at the University of North Carolina at Chapel Hill. His clinical research is focused on the development of new therapies and the vasculopathy of sickle cell disease, with an emphasis on pulmonary hypertension, renal complications and coagulation activation in sickle cell disease. He is presently a Professor of Medicine and Director of the Comprehensive Sickle Cell Program at the University of North Carolina at Chapel Hill, North Carolina, USA.
Sickle cell disease (SCD) is a multisystem disease, associated with episodes of acute illness and progressive organ damage. Despite its low prevalence in the United States, SCD is one of the most common severe monogenic disorders worldwide, with an estimated 230,000 affected children born every year in sub-Saharan Africa (1). As a result of its multiple complications, SCD is associated with an overall decreased life expectancy (2-4). In addition, the economic burden of SCD is enormous - a consequence of recurrent and chronic SCD-related complications, frequent absences from school and work, and physical impairment limiting the ability to contribute to society.

Although there is an increased understanding of the pathophysiology of SCD (5), available pharmacologic treatments remain limited. The current approach to the management of SCD includes supportive treatments with folic acid; symptomatic treatment with analgesics, intravenous fluids, oxygen and red blood cell (RBC) transfusion; disease-modifying treatment with hydroxyurea and RBC transfusion (6-10); and curative therapy with bone marrow transplantation (11). Despite the success of bone marrow transplantation as a curative approach in SCD, this modality is limited by its high cost, decreased availability of suitable donors and toxicity.

Hydroxyurea remains the only drug approved by the US Food and Drug Administration specifically for SCD. The results of several recent large clinical trials evaluating new drugs in SCD have been disappointing (12-15). However, due to an increased interest in the development of new drugs for SCD, several promising therapeutic agents are currently being tested (16). While treatments may target any one of multiple SCD-related complications, most drugs tested to date have been focused on the prevention or termination of acute pain episodes (frequently referred to as vaso-occlusive crises).

In this special issue of the journal, several leaders in the field review novel pharmacologic approaches to the treatment of SCD. Drs. Perrine and Lui provide an overview of the pathophysiology of SCD and the role of inducers of fetal hemoglobin, anti-oxidants, anti-adhesive and anti-inflammatory agents, and drugs that improve red cell hydration. Dr. Wang provides a description of the clinical considerations for the use of hydroxyurea and its effects on organ function. Dr. Wun discusses the evidence for a contribution of platelet activation to SCD-related complications and the studies of platelet inhibition in SCD. Drs. El-Rassi and Morris discuss the association of low bioavailability of arginine and glutamine with SCD-related complications as well as the effects of supplementation with these amino acids. Drs. Sharma, Potoka and Kato describe the possible pathophysiologic role of impaired nitric oxide bioavailability and the effect of therapeutic strategies to restore nitric oxide balance in SCD. Finally, Drs. Gupta, Thompson, Gupta and Abrams review the experimental and clinical evidence to support investigations of the use of cannabis for the treatment of pain in SCD.

References


SECTION I
Pharmacologic Therapies for Sickle Cell Disease
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Experimental Therapeutics for Sickle Cell Disease: Challenges and New Potential

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ABSTRACT

Sickle cell anemia is a serious disease which can cause progressive and widespread organ damage, due to polymerization of sickle hemoglobin and distortion of red blood cells. The sickle globin gene arose independently in 5 global regions, and diverse genetic modifiers influence individual phenotypes. Therapies targeted to the primary pathophysiologic features are needed: anemia, cell adhesion to vasculature, leading to vaso-occlusion, and intravascular hemolysis with nitrite consumption. Treatments for secondary features of inflammation and specific organ complications are also needed. Individual pathology is complex and unique, reflecting organ damage, pain levels, and modifying factors. Proving that any therapeutic is effective in such diverse subjects is challenging. While hydroxyurea benefits many subjects largely by HbF enhancement, others cannot tolerate optimal doses. Additional therapies to achieve higher HbF and F-cell levels would benefit most adolescent and adult patients. Currently, therapeutics which target primary and secondary pathophysiologic features are under development, including small molecule HbF enhancers which target gene co-repressors; L-glutamine, a successful Phase 3 therapeutic which reduces oxidative damage and vaso-occlusive crisis frequency; Vepoloxamer, a rheologic agent which inhibits adhesion interactions and enhances blood flow; selectin inhibitors; inhibitors of hemoglobin polymerization; and anti-inflammatory agents. An ion channel inhibitor, Senicaproc, prevents cell dehydration and reduces hemolysis. Therapies with different actions are needed to control most diseases, a pipeline is cautiously becoming feasible for sickle cell disease.

INTRODUCTION

The sickle globin gene arose independently 5 times in diverse global regions and was selected for because the carrier state conferred resistance to falciparum malaria. However, inheritance of two βs globin genes, or one βs globin gene with a second abnormal β globin gene, produces severe syndromes. The primary pathophysiology is due to anemia, from hemoglobin polymerization which causes reduced red cell survival and low erythropoietin levels, adhesion of abnormal red cells to the vasculature, with compounding adherence of neutrophils, platelets, and coagulation factors, (causing vaso-occlusion and tissue ischemia), and hemolysis. When intravascular red cell lysis is a predominant feature, specific complications related to consumption of nitric oxide result, including pulmonary hypertension, refractory leg ulcers, and priapism. Secondary features include inflammation at sites of vaso-occlusion and tissue damage, and related acute and chronic pain. Therapeutics which reduce all of these features are needed to truly control sickle cell disease and reduce morbidity and early mortality.

For 2 decades, there has been only one FDA-approved therapeutic for treatment of the primary pathology of sickle cell disease, hydroxyurea, which has had great benefit for many patients, particularly at early ages. Most therapeutics are effective in 25-60% of patients, and by all measures hydroxyurea is highly successful as a single agent, reducing vaso-occlusive crises, acute chest syndrome, transfusion requirements, and extending survival, all attributed to its effects on enhancing fetal globin. Its efficacy in reducing cell adhesion and increasing red cell volume, which reduces the intracellular concentration of β globin, contribute to its impact. However, with time and repeated episodes of marrow infarction, older patients often cannot tolerate optimal doses of hydroxyurea, and more agents are solely needed for such a complex disease. Currently, multiple therapeutics are in development, achieving major
milestones in clinical trials, and are addressing many aspects of sickle cell disease. This manuscript reviews small molecule therapeutics in development which were presented at the 2015 9th Annual Meeting of the Foundation for Sickle Cell Research. A table of some therapeutics in development is shown in Table I and Figure 1, below.

Figure 1. Diagram of sickle cell pathophysiology and sites of action of therapeutics in development

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**Therapies targeting sickle pathophysiology**

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**Phase 3 Therapeutics to reduce vaso-occlusive crises**

**L-glutamine, an NAD substrate administered to reduce oxidative damage**

A randomized, controlled trial of L-glutamine (PGLG) was recently completed, which demonstrated marked reduction in the frequency of vaso-occlusive crises, hospitalizations, acute chest syndrome, and days without a crisis. The therapeutic was based on basic science evidence that enhancement of nicotinamide adenine dinucleotide (NAD) modulates oxidation-reduction in sickle red blood cells, and that NAD is enhanced by administering a precursor, L-glutamine. A Phase 3 randomized (2:1), placebo-controlled trial was conducted in 230 patients who had had at least 2 vaso-occlusive crises during the preceding 12 months, in 31 sites. L-glutamine (PGLG) or placebo was given in 2 oral doses at 0.6 mg/kg/day. A highly statistically significant reduction in all study endpoints was demonstrated in L-glutamine-treated compared to placebo, including:

- Vaso-occlusive crises in treated subjects vs placebo subjects, 3 vs 4, and hospitalizations 2 vs 3, \( p = 0.005 \)
- Median hospital days in L-glutamine treated (6.5) vs placebo treated (11) days, \( p = 0.02 \)
- Acute chest syndrome in L-glutamine treated (11.9)% vs (26.9)% \( p = 0.006 \)
- Time to first crisis in L-glutamine treated vs placebo treated, 87 days vs 54 days respectively, \( p = 0.001 \)

These impressive results were consistent within hydroxyurea usage groups, age, and gender. The company, Emmaus Life Sciences Inc., which conducted
the study is now preparing a New Drug Application (NDA) for US FDA approval. It is highly encouraging that a second drug to reduce the most frequent cause and duration of hospitalizations has been clearly demonstrated to be effective. Further, reducing the frequency of acute chest syndrome (ACS) a complication associated with high mortality with a safe therapeutic is highly encouraging. It will be interesting to determine the benefit of combined use of HU and PGL over a longer duration of time.

Vepoloxamer to enhance blood flow in acute vaso-occlusive crises

MAST-188, Vepoloxamer, is currently in a global Phase 3 randomized, double-blind, placebo controlled trial which is projected to complete enrollment by the end of 2015. Formerly termed ANX-188, Vepolamer is a biophysical agent which reduces adhesion of injured cells, inhibits multiple adhesion interactions, rapidly enhances blood flow, (visualized by video microscopy in patients receiving treatment with MST-188), reduces hemolysis, and has already shown significant reduction in duration of sickle cell crises in a previous trial, (from 80 hours mean duration with placebo treatment compared to 44 hours in treated subjects, (p<0.02). The prior Phase 3 trial was discontinued early due to financial and enrollment issues. There were a number of unanticipated difficulties in the endpoints previously employed. For example, agreement on discharge readiness by patient and physician were required but not always in sync, and there were delays simply due to physician schedules.

These attempts were reviewed and revised for practice trends in the current EPIC trial. The drug was reformulated by a new company, MAST Therapeutics, and is given intravenously during hospitalization for an acute vaso-occlusive crisis, with prompt institution within 12 hours of presentation at a hospital facility. Refinements include new primary and secondary endpoints, and more sensitive data capture of opioid use with patient controlled analgesia (PCA) in all subjects, as follows:

- Duration of crisis is defined as time of onset to discontinuation of parenteral opioids, (a meaningful surrogate for resolution of crisis)
- Duration of hospitalization
- Development of acute chest syndrome
- Readmission rate within 14 days

These endpoints were selected after extensive discussion with treating physicians and the FDA, and have guided endpoints for other therapeutic trials for acute crises, already producing a major impact on trial design for acute sickle cell crisis. With positive findings, future use of Vepoloxamer may be expanded to the first signs of a crisis, perhaps with administration in outpatient infusion units. It would be interesting to document, in the future, if long-term damage is reduced by Vepoloxamer, when used to limit duration of sickle cell vaso-occlusive.

Anti-adhesion therapy GMI-1070, Rivipansel

Adhesion of sickle red cells to vascular endothelium also results in adhesion of neutrophils, platelets, coagulation factors, and activation of the endothelium cells, which contribute to vaso-occlusion and secondary inflammation in sickle cell disease. Adhesion is initiated by binding of P-selectin, which is constitutively expressed by endothelial cells and released from activated platelets. Inhibition of P-selectin binding should reduce vaso-occlusion profoundly, and an oral inhibitor is under development. Telen and colleagues reported on a Phase 2 trial in 76 patients of an IV selectin inhibitor, GMI-1070 (Rivipansel), in acute vaso-occlusive crises, administered every 12 hours by IV infusion during a crisis. Because inflammatory cytokines upregulate E-selectin expression, which enhances leukocyte adhesion, GMI-1070 is considered to have significant potential. A 28 and 48% shorter in mean and median time to crisis resolution (41 and 63 hours) was observed in GMI-1070 treated subjects, respectively, compared to placebo-treated control subjects in the Phase 2 trial. Although not statistically significant, the findings are highly clinically meaningful, and the drug is progressing to Phase 3 trials. A secondary endpoint of significantly reduced total IV opioid use with GMI-1070 treatment compared to placebo was a striking, unexpected observation. The highly significant outcome of reduced opioid use offers an objective target for many future sickle cell clinical trials; reduction in pain scores at various time points also was favorable in treated patients, although this is a difficult endpoint with 50% of patients having chronic pain every day of their lives.

Inducers of Fetal Hemoglobin

Enhancement of fetal globin is an established treatment modality which has been demonstrated over decades to ameliorate sickle cell disease in many types of studies; biochemically, to reduce sickle hemoglobin polymerization, as fetal globin chains cannot participate in polymerization; in natural history studies, to reduce clinical severity; in genetic syndromes and populations with naturally occurring elevations in HbF and F-cells, and in therapeutic studies. The impact of HbF is particularly notable in infants with sickle cell disease, who survive in utero in a highly hypoxic environment, which should produce completely sickled cells with no oxygen delivery, if not for the presence of HbF in every red blood cell. Other natural models of elevated HbF include sickle cell populations with milder disease, with >20% HbF and 75% F-cells in the Eastern province of Saudi Arabia, Kuwait, and in India, representing the Arabian Indian
haplotype, (although a small minority of patients still have frequent crises), and in S-HPFH, with 30% HbF with 100% F-cells, an entirely benign condition. The NIH-Cooperative Study of Sickle Cell Disease, and multiple studies of Hydroxyurea (HU), have demonstrated the highly significant ameliorating effects of HbF at levels >8.6% or absolute levels of 0.5 g/dl. Treatment trials, such as with Arginine Butyrate which increased HbF 3-fold, from a mean 7% to 21%, and reduced hospital days by 4-fold, and in severely affected patients treated with decitabine, which reduced cell pathology and increased total Hb and the cardiac biomarker pro-NT-BNP, in a majority of patients. Inducing γ-globin expression by even small increments is a recognized therapeutic approach that should be amenable to broad application, as fetal globin genes are normally integrated in hematopoietic stem cells. Hydroxyurea therapy has increased survival in association with HbF induction. Several important principles for effectively applying HbF induction as a treatment to reduce underlying pathology have been defined:

- HbF may be a cell stress response, which requires some time without the inducing agent.
- Intermittent dosing may be required for many agents, especially if erythroid cell proliferation is inhibited.
- Enhancing translation of fetal globin may be as effective as inducing transcription.
- Patients with higher levels of erythropoietin (EPO) have higher responses to some therapeutics, reflecting erythroid survival actions of EPO, a factor for severely anemic patients who have relative EPO deficiency.

Figure 2. Schema of mechanisms of action of therapeutic candidates on co-repressor complexes or co-activators of the fetal globin gene promoter. Inhibitors of LSD-1 (tranylcypromine, RN-1), DNMT-1 (decitabine), and multiple HDACs disrupt repressor complexes and BCL11A. Treatment with select short chain fatty acids results in displacement of co-repressors (HDAC-2, HDAC-3, LSD-1) and recruitment of EKLF, as an activator, to the γ-globin promoter.

Dover, Bunn, Franco, and others recognized that the proportion of F-cells is important in disease amelioration, and there is selective survival of cells containing HbF compared to non-F cells. Steinberg and colleagues recently hypothesized from mathematical models that both proportions of F-cells and amount of (pg) F/cell are important elements to target for amelioration of most complications. Molecular and cellular mechanisms of fetal globin regulation has been extensively studied, and repressors and activators which modulate gamma globin transcription have been identified, illustrated in Figure 2. Disruption of components of a repressor complex allows interaction of the Locus Control Region (LCR) with the gamma globin promoter, rather than the beta globin promoter. Several small molecule therapeutics have been identified which modulate co-repressors, by chemical inhibition or disrupting protein interfaces between specific co-repressors and can induce gene activation; loss of function of coactivator components can decrease gene expression. Several small molecules which inhibit, or displace, known repressor components are in trials or nearing clinical trials. Decitabine is being repurposed with tetrahydrouridine (THU), an inhibitor of the GI enzyme which metabolizes it rapidly, to enable oral dosing. A combination of agents which create accessible chromatin structure and relieve transcription inhibition may produce profound results. Recent discovery of new therapeutic candidates now offers a renaissance for this approach. The following classes of therapeutics have demonstrated potent effects in preclinical models and in early trials of some agents:

- DNMT-1 inhibition by decitabine in a combination formulation with THU
- HDAC 1,2,3 inhibitors, or displacement of HDAC 2 and 3
- Inhibition of BCL11A, accomplished with arginine butyrate and other histone deacetylase inhibitors, with an oral short chain fatty acid derivative which displaces HDAC-2, (sodium dimethylbutyrate), and by benserazide, a candidate discovered through high throughput screening.
- LSD-1 inhibitors
- Imids- Pomalidamide has greatly enhanced proportions of F-cells in a clinical trial, and has the added benefit of providing anti-inflammatory activity.

Trials of prior generation inducers showed that responses vary in patients with different erythropoietin (EPO) levels, and that intermittent, or pulsed, dosing regimens are superior for at least one therapeutic which inhibits erythroid proliferation, even slightly. While there are clearly established benefit (including survival) of higher HbF levels in sickle cell disease from a wide body of basic and clinical science over decades, a long duration of observation is required to demonstrate survival benefit, and this is not feasible for a trial endpoint. Other endpoints which have been shown with HbF inducers, such as rises in total hemoglobin (Hb), have been accepted for FDA approval of therapeutics for other anemias, with a second benefit such as a quality of life, (such as the Facit fatigue scale) or reduced transfusions. Anemia is related both to the shortened lifespan of sickle red cells and to reduced erythropoietin levels, both increased destruction and decreased production. Higher total Hb levels correlate with reduced fewer silent infarcts and improved cognition. As fatigue is the symptom most commonly reported, by >60% of patients at a recent FDA meeting, reduction of the anemia is clinically important. Anti-adhesion therapeutics are not likely to affect this hallmark complication of sickle cell anemia significantly, and rises in total hemoglobin should be a reasonable endpoint for a HbF-inducing therapeutic candidate in sickle cell anemia.

Topical Nitrite for treatment of sickle cell leg ulcers, a complication of NO depletion

Although many patients have both vaso-occlusive and hemolytic complications, the hemolytic phenotype in sickle cell disease is clearly predominant in some patients, who have high LDH levels and develop refractory leg ulcers, pulmonary hypertension, and priapism. Types of therapies which should benefit this subset include agents to increase total Hb levels and reduce hemolysis (HbF enhancers, Senicaproc, polymerization inhibitors) and specific treatments for leg ulcers, which are debilitating and painful. As hemolysis is considered related to depletion of nitric oxide, topical sodium nitrite to enhance blood flow in leg ulcers and recently was studied in a Phase 2 trial in 18 patients. Application of topical sodium nitrite cream twice weekly was associated with increased peri-wound skin temperature and cutaneous blood flow, documented by thermo-imaging. Reduction in the area of leg ulcers was significantly greater in treated subjects compared to controls (p<0.002), as was pain severity. This appears to be a patient-friendly, safe, and targeted therapy which deserves further investigation.

Other therapeutics

For the first time in decades, a critical mass of therapeutic candidates are in clinical trials or late preclinical testing, including some therapeutics which are already approved for use in other conditions and should have a more rapid route for approval since their safety profiles are known. These include anti-inflammatory agents which act via different mechanisms. Arginine, a substrate of nitric oxide, is being investigated to reduce the duration of acute vaso-occlusive crises, as a substrate to enhance nitric oxide, and IV Gamma Globulin is being investigated to reduce adhesion and neutrophil traps by academic investigators. Agents which both induce fetal globin in animal models and are anti-inflammatory are in early clinical trials.

Challenges of demonstrating efficacy in a serious complex disease: experience with Senicaproc, a designer drug which reduces hemolysis, a cardiac biomarker, and increases hemoglobin

As differences in metabolism alone account for efficacy rates of 25-60% for most major drugs, it is important to adequately power trials and employ endpoints that are feasible to demonstrate in a ill patient population with variable chronic organ damage, different basal levels of pain. Senicaproc is a therapeutic which was designed to reduce the cellular dehydration of sickle cell erythrocytes (which promotes polymerization and all the sickle pathology) through inhibition of a K-CL (ion channel) co-transporter. The therapeutic as evaluated in clinical trials, where treatment increased total hemoglobin (Hb) levels, and strikingly reduced laboratory parameters of hemolysis and a biomarker of cardiac strain (pro-NT BNP) in those subjects whose total Hb rose by >0.05 g/dl. Unfortunately, in a Phase 3 trial, a higher rate of pain crises was observed in treated subjects, and development was stopped early. This therapeutic clearly has a beneficial impact on the pathophysiology of sickle red cells, reduces anemia, and should reduce the serious complications of intravascular hemolysis, for which there is no specific therapy. It is possible that increased blood viscosity, which is hypothesized to occur with higher Hb levels, contributed to the pain episodes. With recognition of fatigue as the most common complaint reported by patients, and now established correlations of CNS complications with greater anemia, a new attempt to apply Senicaproc should be considered, perhaps in combination with hydroxyurea, or with intermittent dosing and dose interruption at a hemoglobin level such as 10 g/dl, a limit generally used for transfusions.

Influence of genetic modifiers in different populations

A number of genetic modifiers, both within and outside the globin locus, have been identified which are associated with higher basal levels of HbF or F-cells, such as the
Xmn-1 single nucleotide polymorphism (SNP) associated with higher Gγ levels, BCL1A, or HMIP, or SNPS associated with higher responses to hydroxyurea such as FOXO3, LAMA5, SAR1, PP1R15A, while AKAP12 was associated with lower responses in the baby HUGS study. The Xmn-1 SNP appears to correlate with responses to sodium 2,2 dimethylbutyrate. Higher basal HbF with some SNPs such as BCL1A, correlate with higher total Hb levels in thalassemia patients with the same molecular mutations. However, the same SNPs have been found to have minor or no effects in one population but high impact in other populations. Efforts are underway to define profiles which are favorable or unfavorable to individual therapeutics, to develop rationale for personalized therapies. Now, after almost 20 years with only one therapeutic approved for treatment of sickle cell disease, a number of therapeutics are showing promise for management of different aspects of sickle cell disease. As response rates are typically 25-60% for most major drugs, setting an approval bar too high, could deprive subsets who can benefit from different candidates. It is important that a dialogue continues between patients, clinicians, pharmaceutical developers, and regulatory authorities, to accelerate approval of therapeutics in the near future, and that investigation continues after approval to expand and optimize therapeutic use in subjects most likely to benefit.

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REFERENCES


Activators/ repressors


SNPs


Other

60. FDA. Voice of the Patient, 2014.


TABLE 1. Classes of therapeutics for treatment of sickle cell disease

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<tr>
<th>Therapeutics which reduce polymerization and anemia</th>
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<tr>
<td>HbF inducers: SCFADs, HDAC, DNMT-1, LSD-1 inhibitors or displacers</td>
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<td>Anti-sickling agents –Aes-103 (HMF)</td>
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<td>Ion channel inhibitors to prevent cell dehydration, (Senicaproc)</td>
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<th>Therapies to reduce the duration of vaso-occlusive crises (VOC)</th>
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<td>Rheologic agent: Restores blood flow - Vepoloxamer</td>
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<td>Adhesion factors: Pan-selectin inhibitors, (eg GMI-1070, Rivipansel)</td>
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<th>Therapies to reduce cell adhesion and vaso-occlusive crises (VOC)</th>
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<td>L-glutamine – Successful Phase 3 trial for acute crisis</td>
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<tr>
<td>P-selectin Antibody, Sel-G</td>
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<td>Arginine HCl</td>
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<td>IV Gamma globulin</td>
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<th>Therapy for complications of NO depletion, related to hemolysis</th>
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<td>Topical nitric oxide for sickle cell leg ulcers</td>
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<td>Therapies for Pulmonary hypertension</td>
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The Potential Role of Platelet Inhibition in the Treatment of Sickle Cell Disease

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Keyword: sickle cell, platelet activation, aspirin, platelet inhibitors

Abstract

The pathophysiology of sickle cell disease is complex. A large amount of data has shown evidence for contribution of platelet activation to sickle cell disease (SCD) related complications. Although some studies of antiplatelet agents in SCD are decades old, there is renewed interest in determining whether platelet inhibition can decrease SCD related complications. This review will provide a brief summary of the evidence for the role of platelets in SCD, and discuss the studies of platelet inhibition.

Introduction

Sickle cell disease (SCD) is the result of homozygous or compound heterozygous inheritance of mutations in the β-globin gene. The resulting substitution of the hydrophilic amino acid glutamic acid at the sixth position by the hydrophobic amino acid valine, leads to the production of hemoglobin S (HbS). HbS polymerizes when deoxygenated and this polymerization is associated with cell dehydration and increased red cell density (1-3). The dense, rigid, sickling and abnormally adherent red cells lead to vaso-occlusion and impaired blood flow (2, 4), and is thought to underlie acute (painful episodes, acute chest syndrome) and chronic (avascular necrosis, renal disease) complications of the disease. Also, intracellular polymerization ultimately damages the red cell membrane and leads to chronic and episodic extravascular and intravascular hemolytic anemia, hemolysis linked nitric oxide dysregulation and endothelial dysfunction (5) resulting leg ulcer, pulmonary hypertension, priapism and stroke (6).

Several investigators have reported alteration in hemostatic system in SCD both under steady state and during acute events. These changes include increased expression of tissue factor on blood monocytes (7-9) and endothelial cells (10, 11); abnormal exposure of phosphatidylserine on the red cell surface (12, 13); platelet activation(14-18); increased microparticles (platelet and red cell derived), which both promote activation of coagulation cascade (19-21); and high incidence of anti-phospholipid antibodies (22, 23).

Contribution of platelets to sickle cell pathophysiology

Platelets might contribute SCD pathogenesis in several ways (Table 1). Hemostasis and inflammation are intimately linked and there are data suggesting an important role for platelets in innate and acquired immunity.(24) The pathophysiology of hypercoagulability in
sickle cell disease is multi-factorial and is a result of alteration in almost every component of the hemostasis system. The reader is referred to other reviews of overall contribution of the hemostatic system(25-27) and herein the focus will be on the contribution of platelets to SCD complications.

Platelet abnormalities (function, number and survival) both when clinically well and during acute events were some of the earliest hemostatic changes documented in sickle cell disease (14, 16, 28-33). Several biomarkers have been measured to document the functional abnormalities. Urinary thromboxane- A2 and prostaglandin metabolites are increased and platelet trombospordin-1 level is decreased in sickle cell disease (34, 35). These findings suggested ongoing platelet activation. Increased platelet activation markers such as platelet surface expression of P-selectin (CD62), CD63, activated glycoprotein (GP)IIb/IIIa, plasma soluble factors (PF)-3, PF4, β-thromboglobulin and platelet-derived soluble CD40 ligand (sCD40L) have been reported in SCD patients(14, 15, 36, 37). Also, platelet adherence to fibrinogen was increased through modulation of signaling pathways associated with increased αIIβ3-integrin activation (38). Platelet aggregation in adults with SCD was increased, perhaps due to increased numbers of megathrombocytes in the peripheral circulation (33, 39, 40) or as a result of increase in levels of platelet agonists, such as thrombin, adenosine diphosphate, or epinephrine. In contrast to adults, platelet aggregation in children has been reported to be normal or reduced, perhaps due to better preservation of splenic function or fewer circulating megathrombocytes (16, 28, 30). Increased phosphatidylserine rich platelets have also been described in SCD patients, which might accelerate the activation of the coagulation system (15).

Platelet number and survival are also abnormal in both steady state and acute events. In steady state there is moderate thrombocytosis in older children and adults with sickle cell anemia (39). The number of circulating megathrombocytes, which are young and metabolically active platelets is also increased. These findings have been attributed to the functional asplenia seen in patients with SCD (40). While studies performed during steady state suggest normal platelet survival (29, 41) decrease in platelet lifespan have been reported in vaso-occlusive crisis (29, 42, 43). Platelet and megathrombocyte counts may decrease markedly, especially when the crisis is severe (31). These decreases are followed by marked rebound increases in platelet and megathrombocyte counts, with levels peaking 10 to 14 days after the onset of the crisis (29, 43).

Celik and colleagues reported on the relationship of mean platelet volume (MPV) to clinical events in Pakistani patients with SCD. Patients were grouped according to the frequency of the crises for the previous year preceding the data collection. Group 1: 1 to 3 crises, Group 2: 4 to 5 and Group 3: 6 or more crises annually. MPV values were found to be higher in patients with cerebrovascular events. Also MPV values increased with increasing incidence of the crises (r=0.297) (p=0.001). The authors speculated the increased MPV was indicative of platelet activation and/or splenic dysfunction.

All of these findings suggest that both shortened platelet survival and enhanced platelet consumption occur during vaso-occlusive crises, possibly because platelets are being deposited at sites of vascular injury or vascular occlusion. It has been demonstrated that labeled platelets accumulate at the putative sites of vaso-occlusion (42).

Polymorphisms in human platelet alloantigen genes may determine, in part platelet reactivity and have been associated with variable risk of thrombotic events, mostly arterial (44). Studies on polymorphisms of human platelet alloantigen (HPA) show a possible pro-thrombotic role in different thrombotic disorders and in sickle cell patients with cerebrovascular events (44-48). In a case-control study, Al-Subaie et al. reported that the HPA-3 variant which has an isoleucine-to-serine substitution close to the C-terminus of the GPIIb heavy chain was an independent risk factor for acute vaso-occlusive events in SCD (48).

Activated platelets could contribute to SCD pathophysiology through at least two pathways: the hemostasis/coagulation cascade and inflammation. Platelets play a central role in hemostasis from primary adhesion, secretion of pro-thrombotic proteins and platelet-derived microparticles, to provision of the anionic phospholipid surface for assembly of the proteases complexes of the coagulation cascade. Many investigators have demonstrated increases in various biomarkers of thrombin generation in patients with SCD(25-27). There are also clinical studies that suggest anticoagulant therapy with heparin may improve the outcome in vaso-occlusive crisis(49).

There is also increasing evidence that platelets play an important role in inflammation and specifically vascular inflammation(24). Increased platelet-monocyte(50-52)and platelet-neutrophil aggregates(51-53) have been demonstrated in SCD patients with attendant increase in neutrophil(54)and monocyte(7) activation. Leukocyte activation likely mediates, at least in part, complications of SCD(55-58).

Platelet activation not only contribute to the classic complications associated with SCD, but also likely play a role in emerging data that SCD patients have increased risk for thromboembolic disease. Although
increased ischemic stroke in sickle cell disease is most often attributed to large vessel arterial remodeling and stenosis, superimposed thrombosis has also been described (59). New and old thrombi in the pulmonary vasculature are prevalent in autopsy series (60-62). The analysis of a large discharge database found that the incidence of pulmonary embolism was 50-fold to 100-fold higher in the SCD population (0.22–0.52%) than in the general population (0.0039–0.0058%)(63). A retrospective study of reported discharge diagnoses showed that patients with SCD younger than 40 years were more likely to be diagnosed with pulmonary embolism compared to African Americans without SCD (0.44% vs. 0.12%)(64). In another retrospective study of 404 sickle cell patients, 25% of the patients had a history of venous thromboembolism (VTE; 18.8% non-catheter related). Sickle cell variant genotypes, such as HbSC or HbSβ+ thalassemia, were associated with increased risk of VTE compared to HbSS. Non-catheter-related VTE was an independent risk factor for death in adults with sickle cell disease (65).

Studies of Platelet Inhibition in Sickle Cell Disease

Given the data for the potential contribution of platelets to sickle cell pathophysiology, it is possible that platelet inhibition might ameliorate or prevent sickle cell related complications. Platelet inhibitors are used in cardiovascular disease with known good safety profile in a wide variety of patient populations. Thus, there is sound rationale for clinical trials examining platelet inhibition in SCD.

There are only three studies in humans evaluating the therapeutic effect of aspirin in sickle cell disease (Table II). These studies were conducted in 1980s and conclusions are limited by the study design. Osano et al. investigated the therapeutic effect of aspirin in 100 patients with homozygous SCD aged 11-20 years (66). In this study patients were randomized to receive total daily dose of 1200 mg aspirin for 6 weeks or placebo in addition to usual care. Hemoglobin levels and oxygen saturation increased in the aspirin arm with increased red cell survival in the 3 patients in whom red cell survival was measured. There were no serious hemorrhagic events in the treatment group. Pain was not formally assessed as an outcome measure in this study. However, in another double-blind placebo-controlled cross-over study of a lower dose of aspirin (3-6 mg/kg) for longer period of time (21 months) in 49 children with HbSS, HbSC, or HbSβ-Arab aged 2-17 years, there was no difference in the number of painful episodes, number of total days in pain, duration of pain crisis, or pain severity during crisis between the aspirin and placebo treated periods. In both the aspirin and placebo arm of this study, there was a marked decrease in the number of pain crises after the first 6 months(67). Similarly, a single-blind crossover study of 29 patients ages 4-31 years receiving 17-45 mg/kg/day of aspirin for five months followed by no aspirin for the next five months (68), did not find difference in the painful events between the periods on and off active therapy.

Dipyridamole is a phosphodiesterase inhibitor used in patients with peripheral vascular disease. Chaplin and colleagues treated 3 patients with aspirin 650 mg PO twice a day and dipyridamole 50 mg PO twice daily for acute pain crisis and compared the frequency and severity of pain for the 2 years on therapy to the next 2 years off the therapy (69). The severity of pain and the total number of hospitalizations for pain decreased. During the study there was no evidence of increased bleeding.

Thienopyridines inhibit platelet function by blocking the interaction of ADP with the platelet surface receptor P2Y12(70). Small studies of the thienopyridines ticlopidine, clopidogrel, and prasugrel have all been conducted in patients with SCD. Semple and colleagues assessed platelet survival and activation in 9 asymptomatic patients with SCD (42) who were randomly assigned to placebo or ticlopidine 250 mg PO twice daily. Ticlopidine did not prolong platelet survival (measured by radio-labeled platelets) but 40% reduction in collagen and ADP-induced maximal platelet aggregation was observed in this double-blind placebo-controlled trial. One patient had a painful episode during the therapy, but this study was not powered to determine a difference in pain. Cabannes et al. randomized 140 SCD patients to ticlopidine 500 - 750 mg daily for 6 months or placebo with pain crisis the primary outcome measure(71). Frequency of crisis, crisis duration, and crisis severity decreased in the ticlopidine arm compared with the placebo arm. More recently, Wun et al (72) studied the third generation thienopyridine prasugrel in a randomized, double blind adaptive Phase 2 study in adults with all genotypes of sickle cell disease. Patients were randomized to prasugrel 5 mg daily (n=41) or placebo (n=21) for 30 days. Platelet function was significantly inhibited in prasugrel compared with placebo treated SCD patients. Biomarkers of in vivo platelet activation, including platelet surface P-selectin and plasma soluble P-selectin, were significantly reduced in SCD patients treated with prasugrel compared with placebo. Mean pain rate (percentage of days with pain) and intensity decreased in the prasugrel arm but did not reach statistical significance. Prasugrel was well tolerated and not associated with serious hemorrhagic events. Despite the small size and short duration of this study, there was a decrease in platelet activation biomarkers and a trend toward decreased pain. Based upon this study and a safety trial performed in children, an international Phase 3 study of prasugrel in children with sickle cell disease is currently enrolling (ClinicalTrials.gov Identifier: NCT01794000). A Phase 2 dose-finding study of the direct acting P2Y12
ticagrelor is also currently enrolling patients (ClinicalTrials.gov Identifier: NCT02214121).

Summary
Although the pathogenesis of sickle cell disease lies in dysfunctional hemoglobin structure and function, platelet activation is a prominent feature in patients with SCD. Biomarker studies of platelet activation suggest that effective platelet inhibition can be achieved in SCD patients, and provocative data from pilot studies indicate there may be improvement in clinically important outcomes. Therefore, clinical trials of antplatelet therapies are justified with both SCD related complications (VOC, pain) and thrombotic complications as outcome events of interest and are currently being done in children. If results do show improvements in sickle cell related complications with acceptable toxicity in this patient population, platelet inhibition would represent a relatively simple and widely available therapy to decrease the morbidity associated with sickle cell disease.

Table 1. Potential Roles of Platelets in Sickle Cell Pathophysiology

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation of the coagulation system</td>
<td>Increased concentration of biomarkers of coagulation system activation</td>
</tr>
<tr>
<td>Enhanced sickle red blood cell adhesion</td>
<td>Secreted platelet granule products mediate red cell adhesion</td>
</tr>
<tr>
<td>Enhanced heterotypic cell-cell interactions</td>
<td>Increased platelet-red cell aggregates</td>
</tr>
<tr>
<td></td>
<td>Increased platelet-monocyte and platelet-neutrophil aggregates</td>
</tr>
<tr>
<td></td>
<td>Neutrophil-platelet-red cell adhesion</td>
</tr>
<tr>
<td>Activation of immune cells and inflammation</td>
<td>Effect of platelet activation on T and B cell immune response</td>
</tr>
<tr>
<td></td>
<td>(not sickle cell specific)</td>
</tr>
<tr>
<td></td>
<td>Induction of B cell proliferation</td>
</tr>
</tbody>
</table>

Table II. Studies of Platelet Inhibition in Sickle Cell Disease

<table>
<thead>
<tr>
<th>Author</th>
<th>Genotypes</th>
<th>Study Type (N)</th>
<th>Therapy</th>
<th>Overall Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaplin et al(69)</td>
<td>HbSS</td>
<td>Non-randomized cross-over (3)</td>
<td>Aspirin and Dipyridamole</td>
<td>Decrease in pain frequency, platelet count and fibrinogen</td>
</tr>
<tr>
<td>Osamo et al(66)</td>
<td>HbSS</td>
<td>Randomized (100)</td>
<td>Aspirin</td>
<td>Increase in oxygen affinity, Hb, and red cell life span</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pain not formally assessed</td>
</tr>
<tr>
<td>Greenberg et al(67)</td>
<td>HbSS/SOArab/SC</td>
<td>Randomized (49)</td>
<td>Aspirin vs. placebo</td>
<td>No decrease in pain frequency</td>
</tr>
<tr>
<td>Semple et al(42)</td>
<td>HbSS/Sβ thalassemia</td>
<td>Randomized (9)</td>
<td>Ticlopidine vs. placebo</td>
<td>No change in pain, but decrease in platelet activation biomarkers</td>
</tr>
<tr>
<td>Cabannes et al(71)</td>
<td>HbSS</td>
<td>Randomized (140)</td>
<td>Ticlopidine vs. placebo</td>
<td>Reduction in frequency and duration of VOC</td>
</tr>
<tr>
<td>Zago et al(68)</td>
<td>HbSS/Sβ thalassemia</td>
<td>Randomized (29)</td>
<td>Aspirin vs. Placebo</td>
<td>No change in pain episodes or laboratory values</td>
</tr>
<tr>
<td>Wun et al(72)</td>
<td>HbSS/Sβ thalassemia/SC</td>
<td>Randomized Phase 2 (62)</td>
<td>Prasugrel vs. Placebo</td>
<td>Decrease in platelet activation and trend to decreased pain frequency and rate</td>
</tr>
</tbody>
</table>

Adapted from Ataga and Key(79)
References


Arginine and Glutamine: The Future of Amino Acid Therapy in Sickle Cell Disease

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Abstract
Sickle cell disease (SCD) is an arginine deficiency syndrome. Normal arginine metabolism is impaired through various mechanisms that contribute to endothelial dysfunction, vaso-occlusion, pulmonary complications, risk of leg ulcers and early mortality. Arginine is a semi-essential amino acid that serves as a substrate for protein synthesis, and is the precursor to nitric oxide (NO), polyamines, proline, glutamate, creatine and agmatine. Since it is involved in multiple metabolic processes, an arginine deficiency has the potential to disrupt many cellular and organ functions. NO is a potent vasodilator that is depleted in SCD. As the obligate substrate for NO production, arginine plays a crucial role in endothelial function. Glutamine is also a conditionally essential amino acid found in dietary protein that can be metabolized to arginine and may function as an arginine pro-drug. Since low glutamine and arginine bioavailability are associated with a number of SCD-related complications, supplementation with these deficient amino acids represent promising treatment options for SCD. Clinical studies of arginine therapy in SCD demonstrate efficacy in treating patients with leg ulcers, pulmonary hypertension risk, and acute vaso-occlusive pain. Co-administration of arginine with hydroxyurea increases levels of nitrite and fetal hemoglobin. Oral glutamine supplementation significantly reduced incidence of SCD crises requiring hospitalization, median time to first crisis, episodes of acute chest syndrome and length of hospital stay for patients with SCD requiring admission compared to placebo in phase 2 and phase 3 trials. Restoration of L-arginine bioavailability through exogenous supplementation of L-arginine and L-glutamine is therefore, a promising therapeutic target.

Introduction
Sickle cell disease (SCD) affects nearly 100,000 people in the US and millions worldwide. SCD is caused by a genetic mutation leading to an amino acid substitution of valine for glutamic acid in the sixth position of the β-subunits of the hemoglobin molecule. Under hypoxic conditions, the deoxygenated hemoglobin molecule locks into an irreversible position leading to increased erythrocyte rigidity, damage and distortion of the erythrocyte membrane. This process is often referred to as sickling and it occurs when these polymers becomes entrapped in the circulation, clinically presenting as vaso-occlusive episodes. SCD manifests with a wide spectrum of clinical presentations and degrees of severity that vary from patient to patient; yet, all phenotypes have an element of anemia, pain crises, and potentially life-threatening complications such as bacterial sepsis, splenic sequestration, acute chest syndrome (ACS), stroke and chronic organ damage. These clinical complications are a result of the various mechanisms of interaction of these vaso-occlusive processes, endothelial dysfunction and tissue damage occurring during hemolysis (1-4).
Endothelial dysfunction occurring in SCD is linked in part, to low nitric oxide (NO) bioavailability. Vascular endothelial cells generate NO synthesized directly from the amino acid L-arginine, its obligate substrate via NO synthase (NOS) enzymatic activity. In SCD, NO deficiency occurs as a result of its decreased production and increased consumption (5). Normal arginine metabolism is also impaired through various mechanisms that contribute to endothelial dysfunction, vaso-occlusion, pulmonary complications, risk of leg ulcers and early mortality (6-14). Glutamine is a conditionally essential amino acid naturally found in dietary protein that can be metabolized to depletion is also associated with pulmonary hypertension risk in SCD (18), a serious complication linked to early mortality (19-22). Since low glutamine and arginine bioavailability are associated with a number of SCD-related complications (6, 7, 23), supplementation with these deficient amino acids represents promising treatment options for SCD (6).

Arginine and Global Arginine Bioavailability

Arginine is a semi-essential cationic amino acid involved in multiple pathways in health and disease. It becomes essential however, under conditions of stress and catabolic states when the capacity of endogenous arginine synthesis is exceeded, including trauma, sepsis, burns, and in particular, SCD. Arginine serves as a substrate for protein synthesis, and is the precursor not only to NO, but also polyamines, proline, glutamate, creatine and agmatine. Since it is involved in multiple metabolic processes, an arginine deficiency has the potential to disrupt many cellular and organ functions (9). Arginine is derived from dietary protein intake, body protein breakdown or endogenous de novo arginine production in the kidneys. The majority of whole-body arginine synthesis in adults is performed in a metabolic collaboration by the small intestines and kidneys in what has been termed the “intestinal-renal axis” (15). Dietary glutamine plays an important role in this process, as 90% of circulating citrulline arises from glutamine (24-26). Intestine-derived citrulline is released into the circulation and taken up primarily by the kidneys for arginine transport and all NOS isozymes. Circulating ADMA levels are elevated in several conditions of endothelial dysfunction, including SCD, and are also linked to increased mortality (14).

Sickle Cell Disease and the Arginine-Nitric Oxide Pathway

NO is a potent vasodilator for which arginine is the crucial substrate that leads to NO synthesis. NOS metabolizes L-arginine first to the intermediate N-hydroxy-L-arginine (NOHA) to form NO and L-citrulline. Several co-factors are necessary for normal NOS function in addition to adequate arginine bioavailability, including oxygen, nicotinamide adenine dinucleotide phosphate (NADP), tetrahydrobiopterin, and sufficient glutathione availability (34, 35). Of the many functions NO possesses, the effects particularly relevant to SCD are extensive and range from...
decreasing platelet activation (36) and adhesion receptor expression on the vascular endothelium, to decreasing vascular smooth muscle proliferation (37), limiting ischemia-reperfusion injury (38), modulating endothelial proliferation (3), and regulating inflammation (39). Researchers have found that NOS expression and activity are increased in SCD (40); yet NO bioavailability is paradoxically decreased (41, 42). Work by Hsu and colleagues in the sickle cell transgenic mouse model demonstrated that NOS is uncoupled and therefore dysfunctional, producing superoxide in lieu of NO, which would rapidly react with NO to produce peroxynitrite, a potent oxidant responsible for cell damage and cell death (40). Impaired NO bioavailability in this disorder is further demonstrated by a blunted response to endothelium-dependent vasodilators in the sickle cell mouse model, (43, 44) as well as reduced flow-mediated vasodilation in patients with SCD (45-47). Additionally, NO metabolites (NOx) are noted to be elevated in patients with SCD at steady-state compared to normal controls (48), while peripheral vascular resistance and resting blood pressures are low. This likely represents a compensatory response in SCD that is overwhelmed in acute states of crisis. NOx levels drop significantly during times of stress including vaso-occlusive pain episodes (VOE) (13, 49) and ACS (13), varying inversely with degree of pain (50).

Where does arginine fit into the picture presented thus far? Arginine being the main substrate for the NO pathway is deficient at steady-state (13, 51, 52) in adults with SCD, whereas children have plasma levels that are similar to normal controls (13). This state of arginine deficiency is influenced by acute phases of the disease (13). Despite this noted difference between children and adults, the plasma levels of arginine decrease significantly in both populations during VOE and ACS, and are associated with low NOx levels (13, 53, 54). While recovering from the acute sickling process, levels of arginine and NOx increase back to their baseline levels. Low plasma arginine level was found to be predictive of need for hospital admission in children with SCD and pain presenting for emergency care, while no such correlation was found with NO levels (13), suggesting a role for arginine bioavailability during pain events that goes beyond that of NO production.

Arginine dysregulation in SCD occurs as a result of increased destruction, decreased production and intercellular transport anomalies (6, 12). The uptake of arginine into the cells occurs via the cationic amino acid transporter (CAT) protein which is also responsible for ornithine and lysine uptake. Plasma arginine levels in adults with SCD are approximately 40-50 μM at baseline, low compared to normal controls (80-100 μM) and well below the affinity constant (Km) for the CAT protein (100-150 μM).

Accordingly, even mild fluctuations in extracellular arginine concentration may significantly impact cellular arginine uptake and bioavailability. Also, the fact that both ornithine and lysine share the same CAT protein with arginine makes them obligate competitive inhibitors (6). As mentioned earlier, intracellular arginine transport can be further compromised due to elevated ADMA, a competitive inhibitor of arginine transport known to be increased in SCD (14).

Excess arginase activity is the hallmark of the arginine-deficient state of SCD. The NOS and arginase enzymes can be expressed simultaneously under a wide variety of inflammatory conditions, resulting in competition for their common substrate (55). Arginase is a urea cycle enzyme that catalyzes the hydrolysis of L-arginine to urea and L-ornithine. Both Arginase I and II isoforms are found in many cell types and are constitutively expressed in the human airways; Arginase I is cytosolic and highly expressed in the liver, while arginase II is mitochondrial and extra-hepatic. Arginase-1 is also present in human erythrocytes, which has significant implications for hemolytic disorders like SCD, where it is aberrantly released into plasma in active form as the red blood cells rupture (15). Plasma arginase activity is elevated in SCD as a consequence of inflammation, liver dysfunction and, most significantly, by the release of erythrocyte arginase during intravascular hemolysis (7). Arginase will redirect the metabolism of arginine to ornithine and the formation of polyamines and proline, which are essential to form smooth muscle cell growth and collagen synthesis. This shift toward ornithine metabolism leads to a process that contributes to vascular smooth muscle proliferation and airway remodeling. These are features of asthma and pulmonary hypertension, common co-morbidities found in SCD that are also associated with increased mortality risk and low arginine bioavailability (7, 11, 31).

Hemolysis: An Important Cause of Arginine Dysregulation
Hemolysis is a major manifestation of SCD that can lead to endothelial dysfunction (12, 30, 56-59). This will increase arginine consumption due to release of erythrocyte-arginase from the hemolyzed cells. Hemoglobin is decompartmentalized from the erythrocyte during the process of hemolysis and released into plasma where it rapidly reacts with and destroys NO (41). This results in abnormally high NO consumption, the formation of reactive oxygen species, and a state of NO resistance (42). The simultaneous release of erythrocyte-arginase, will metabolize arginine during hemolysis (7) and further diminished NO bioavailability (Figure 1). Formation of superoxide from enzymatic oxidases such as NADPH oxidase, xanthine oxidase (60) and uncoupled endothelial NOS (40) will also react with and scavenge NO, further amplifying a state of NO resistance. Consequently, smooth muscle guanylyl cyclase is not activated and vasodilation is inhibited. NO destruction by hemoglobin can also cause further impairment in vascular endothelial function via transcriptional activation of adhesion molecules, and potent vasoconstrictors such as endothelin-1 (42). Intravascular hemolysis also has the potential to drive a pro-coagulant state, as NO has properties that inhibits platelet activation, tissue factor expression and thrombin generation (42).

Heme is also released during erythrocyte hemolysis, and has prooxidant, cytotoxic and inflammatory effects. In SCD, extracellular heme has been found to activate the innate immune response and trigger the release of neutrophil extracellular traps (NETs) that promote lung injury. This drives sterile inflammation through heme- Toll-like receptor 4 activation (61). Thus erythrocyte hemolysis by-products can be considered damage-associated molecular pattern molecules (DAMPS) and can contribute to widespread systemic inflammation in the absence of infection. (59)

Arginine Therapy

Although mechanisms of arginine dysregulation are complex and multifactorial (12), they can be overcome through arginine supplementation (8, 62). The exact mechanisms responsible for the benefits of arginine therapy in SCD remain unknown but likely are not limited to NO production alone. In transgenic mouse models of SCD, L-arginine supplementation inhibits the red cell Gardos channels (63), reduces red cell density, improves perfusion, and reduces inflammation (64), lung injury, microvascular vaso-occlusion and mortality (40, 65, 66). Arginine also increases erythrocyte glutathione levels in both mouse (65) and human trials (67). Although the role of NO in SCD has generated some controversy (68, 69), these studies further demonstrate that the mechanistic impact of arginine may go beyond NO production.

Rapid healing of leg ulcers was reported with oral (16) and intravenous arginine-butyrate in both SCD and thalassemia (70). A randomized controlled phase-2 trial of intravenous arginine-butyrate for patients with SCD and chronic recalcitrant leg ulcers confirmed the initial anecdotal observations (71). Short term arginine therapy improved pulmonary hypertension in SCD (16), and acutely increased both plasma and exhaled NO when administered to ethnically matched normal controls and patients hospitalized for pain (53, 72). When arginine is given to SCD patients at steady-state, a paradoxical decrease in NOx occurs that is not overcome by higher doses (53), clearly indicating that arginine is metabolized differently in SCD compared to control subjects. However when arginine is given during VOE, a robust dose-dependent increase in NOx is observed. (53) This indicates that arginine is also metabolized differently in SCD at steady-state compared to times of acute illness including pain and ACS (13, 53, 72). These early observations may account for the negative outcome of the Comprehensive Sickle Cell Centers’ (CSCC) prophylactic arginine trial (73), particularly since the primary outcome measure of that study was an increase in plasma NOx levels, when published data in fact demonstrated a decrease in NOx with arginine supplementation in SCD patients at baseline (53). Ultimately nutritional therapies like arginine may possess the greatest benefit potential during a deficient state.

The capacity of arginine supplementation to increase NOx production in SCD during VOE is dose-dependent (53). Low dose arginine therapy is therefore likely to be sub-therapeutic in SCD, and may represent an additional flaw in the CSCC prophylactic arginine trial design (73), as doses used were close to placebo based on the cardiovascular literature (74, 75). Previous studies have shown that low-dose arginine is unlikely to impact NO synthesis (75), an observation confirmed in the CSCC study (73). Higher levels of plasma arginine are likely needed to overcome multi-factorial effects including impact of arginase and ADMA on global arginine
bioavailability, and accelerated arginine consumption during pain events compared to baseline (6). However, the long-term safety of doses greater than 100 mg/kg/dose given 3 times a day is unknown in SCD, although a 1-time dose of 30 grams IV is safe and commonly used for growth hormone stimulation testing (76).

Based on preliminary pharmacokinetic studies (53, 72), peak plasma arginine concentration after oral arginine (100 mg/kg) is significantly higher during SCD steady-state compared to patients experiencing VOE, although levels are similar by 4 hours. Normal controls reach a peak arginine level between 1-2 hours that is maintained at 4 hours, and does not trend down as in SCD (53). Accelerated arginine metabolism or consumption occurs during pain events compared to steady-state despite the same oral arginine dose given. Similar observations were made with respect to arginine pharmacokinetics in moderate compared to severe malaria (77, 78), suggesting that a greater consumption of arginine may occur when the disease state or hemolytic rate is more severe. An arginine infusion significantly improved endothelial function and maintained plasma L-arginine concentration above the Km for CAT-1 for the duration of the infusion compared to bolus dosing in patients with malaria (77, 79). Bolus dosing provided concentrations above the K for 50% of the patients at 2 hours, and only 25% at 3 hours. It remains to be determined if arginine infusions are superior to bolus dosing of L-arginine in SCD, however this question will be addressed in future clinical trials in SCD that are underway at our institution.

Co-administration of oral arginine with hydroxyurea (HU) ameliorated the paradoxical decrease in plasma NOX observed in patients with SCD at steady state compared with arginine monotherapy (72). A recently published study performed in Brazil adds to the growing body of literature in support of arginine co-administration with HU. Twenty-one adult patients with SCD were randomized to receive HU alone (500–1500 mg/d; n = 9) or HU+arginine (250 mg/d; n=12) for 12 weeks. An increase in levels of nitrite and fetal hemoglobin were observed in the arginine/HU arm compared with patients receiving HU alone (80), despite the low dose of arginine used. Arginine therapy together with HU may be superior to either single intervention. This is important information to consider when designing clinical trials, particularly since up to 50% of patients with Hb-SS may be on HU therapy. Stratification by HU use is important; however patients on HU should not be excluded from arginine trials.

Arginine therapy is promising for pain management of VOEs, the most common reason for emergency department (ED) visits, and hospitalizations, and are associated with an increased mortality rate (81). We have now completed a single-center randomize, double-blinded, placebo-controlled trial of arginine therapy in children with SCD and pain requiring hospitalization (8). Thirty-eight children with SCD admitted for 56 episodes of VOE were randomized to receive oral or parenteral L-arginine (100 mg/kg 3 times per day) or placebo for 5 days or until discharge, whichever occurred sooner. A significant reduction in total parenteral opioid use by 54% (1.9±2.0 mg/kg vs 4.1±4.1 mg/kg, p=0.02) and lower pain scores at discharge (1.9±2.4 vs 3.9±2.9, p=0.01) were observed in the treatment arm receiving arginine compared with placebo (Figure 2). There was no significant difference in hospital length of stay, although a clinically relevant 17-hour reduction trend favored the arginine arm, and total opioid use (mg/kg) correlated strongly to length of admission (p<0.0001; Figure 3). These data suggest that total opioid use may be a useful surrogate biomarker for length of hospital stay in clinical trials. In future studies, delivering arginine therapy as early as possible in the ED or clinic may have a greater impact on time to pain crisis resolution because many patients in the above arginine study received their first dose of study medication more than 24 hours after presenting to the ED in pain (8). No drug-related adverse events were observed. One patient experienced clinical deterioration associated with ACS requiring emergent transfusion and a transfer to the pediatric intensive care unit (PICU) in the placebo arm. No clinical deterioration or PICU transfers occurred in the arginine arm (8). Although a large-scale multicenter trial is needed to confirm these promising observations, arginine may be a beneficial adjunct to standard pain therapy for VOE that could reduce suffering and improve emergency care for children with SCD.

**Glutamine Therapy**

Glutamine is the most abundant amino acid in the body, comprising approximately half of the free amino acids in the blood and muscle. As a non-essential amino acid, glutamine can be produced in the body by conversion from another amino acid, glutamic acid (primarily by the skeletal muscle and liver). Glutamine’s main functions in
the body include serving as a precursor in the synthesis of other amino acids and glucose for energy. It is also the precursor of nicotinamide adenine dinucleotide (NAD), therefore possessing an antioxidant role as well. Cells of the immune system, the small intestine and the kidney are the major consumers of glutamine. Glutamine is naturally found in dietary protein. Like arginine, it is also available without prescription as a dietary supplement. As mentioned previously, it can also be metabolized to citrulline and subsequently to arginine, the amino acid substrate for NO production that becomes deficient in hemolytic conditions.

Glutamine has been extensively study and is considered a very safe supplement administered both orally and intravenously (24, 25, 82-84). It is commonly used in many clinical scenarios including trauma, the intensive care unit setting, burns, low birth-weight infants and in conditions that involve gut inflammation (85-91). Glutamine-enriched diets showed good overall tolerance, improvement of immunologic aspects in multiple trauma patients, cost reduction in critically ill patients, and improvement of mucositis in post-chemotherapy patients. The doses given and the duration of therapy varied widely depending on the pathologic condition. Intake of 20 to 30 g/d are recommended (89) and well tolerated. Over the last 10 years, clinical trials of glutamine supplementation in critical illness, surgical stress, and cancer have shown benefit with regard to mortality, length of stay, and infectious morbidity. No evidence of harm has been observed in studies conducted to date; thus, further clinical trials using glutamine as a pharmacologic supplement to standard nutrition are felt to be warranted. However, data demonstrating a lack of benefit with glutamine supplementation in some patients have been presented as well. It appears that dose and route of administration clearly influence the benefit observed from glutamine administration, with high-dose glutamine demonstrating an advantage over low-dose glutamine (30 g/day enterally) (90). This is also true with arginine supplementation, as low doses are often essentially ineffective in providing clinical benefit (75). Dr. Niihara and colleagues have found glutamine therapy to be beneficial in SCD, utilizing a dose of 30 grams a day without adverse events. These studies demonstrated improvement in NAD redox potential in all SCD patients investigated. In addition, there were consistent reports of improved general clinical condition in such areas as energy level and chronic pain levels (92-94). Furthermore, a short observation period of approximately 3 months suggested a decrease in the incidence of vaso-occlusive painful crises among those patients in preliminary trials. In both cross-sectional and longitudinal studies, these authors demonstrate that L-glutamine therapy improves the endothelial adhesion of sickle red blood cells. Since low erythrocyte glutamine bioavailability is associated with severity of pulmonary hypertension risk in patients with SCD (18), glutamine therapy may decrease oxidative stress and hemolysis as well. Promising results of a phase 3 randomized placebo-controlled trial of L-glutamine for SCD were recently presented at the 2014 American Society of Hematology annual meeting. A total of 230 patients with SCD were treated with L-glutamine (0.6 g/kg/day divided into 2 doses, max 30 g/day) or placebo for 48 weeks, 152 assigned to the glutamine arm, and 78 on placebo. The median incidence of sickle cell crisis was significantly lower in the treatment group compared to the placebo group (3 events vs. 4 respectively; p=0.008); The median incidence of hospitalization was significantly lower in the treatment group compared to placebo group (2 events vs. 3 events respectively; p=0.005); Median cumulative hospital days were significantly lower by 41% in the treatment group (6.5 days) compared to the placebo group (11 days) (p=0.022); 11.9 % of the L-glutamine group and 26.9% of the placebo group were affected by acute chest syndrome (ACS) (p=0.006). The median time to first crisis was 54 days in placebo group and 87 days in treatment group (p=0.010) (95). These results are impressive, especially since glutamine is a nutritional supplement.

**Conclusion**

Amino acid therapy in SCD is a promising field for further exploration and clinical application. Mechanistically, low arginine levels are associated with acute pain, pulmonary hypertension, leg ulcers and early mortality (6-8, 16). Low erythrocyte glutamine levels are associated with pulmonary hypertension risk (18). However, failure of other NO-based therapies in SCD, including the use of inhaled NO for treatment of sickle-related pain (96), and sildenafil for the treatment of pulmonary hypertension (97) has dampened enthusiasm for this therapeutic approach. Nevertheless, promising data from phase-2 randomized controlled trials for arginine treatment of chronic refractory leg ulcers (71) and vaso-occlusive pain in patients with SCD (8) and positive phase-
2 and phase-3 trials of oral L-glutamine supplementation (93, 95, 98) support the future use of these nutritional interventions. L-arginine is a safe and efficacious intervention with narcotic-sparing effects in pediatric SCD patients with acute pain, while L-glutamine significantly reduces the incidence of sickle cell crises requiring hospitalization. However metabolism of arginine by arginase may limit its potential to maximally impact NO synthesis (6, 12). Since glutamine serves as a precursor for the de novo production of arginine through the citrulline-arginine pathway (15, 25, 28), it represents a novel therapy for hemolysis-associated arginine-NO dysregulation that may bypass at least a portion of arginase metabolism. Experience with both arginine and glutamine therapy in SCD has been growing over the last decade (16, 53, 65, 72, 99, 100). No serious adverse events have been reported and extensive safety data has been maintained with the United States Food and Drug Administration. Interventions that target underlying mechanisms of sickle-related pain beyond simply providing symptomatic relief are ideal and are underway.

Figure Legends

Figure 1. Altered arginine metabolism in hemolysis. Dietary glutamine serves as a precursor for the de novo production of arginine through the citrulline-arginine pathway. Arginine is synthesized endogenously from citrulline primarily via the intestinal-renal axis. Arginase and NOS compete for arginine, their common substrate. In sickle cell disease, bioavailability of arginine and nitric oxide (NO) are decreased by several mechanisms linked to hemolysis and oxidative stress. Endothelial dysfunction resulting from NO depletion and increased levels of the downstream products of ornithine metabolism (polyamines and proline) likely contribute to the pathogenesis of lung injury, fibrosis and pulmonary hypertension. This disease paradigm has implications for all hemolytic processes. Reproduced with permission (12).

Figure 2. Impact of arginine therapy on total opioid use (mg/kg) and pain scores in children sickle cell disease hospitalized for vaso-occlusive pain. A. Arginine supplementation (unfilled circles) led to a significant and clinically relevant reduction in total opioid use by 54% over the course of the hospital stay compared to total opioid use in the placebo group (filled circles). The difference remains significant even when the 2 outliers with the largest total opioid use in the placebo arm are excluded from the analysis (p=0.04). B. 10-cm visual analog scale (VAS) pain scores were similar at the time of admission in both groups, but were significantly lower at discharge in the arginine group compared to placebo by 2 cm (p=0.01). Reproduced with permission (8).

Figure 3. Pearson correlation between total opioid use (mg/kg) and total length of hospital stay (days). Total opioid use (mg/kg) is directly correlated to length of hospital stay (r=0.86, p<0.0001). Total opioid use may be a surrogate for length of hospital stay as an outcome measure for patients with SCD and pain. Reproduced with permission (8).

References


Figure 1

Glutamine → Citrulline → Citrulline → ARGININE

Inflammatory cytokines → ↑ NOS → ↓ NO

↑ NO CONSUMPTION:
- HEMOLYSIS: Cell-free Hb
- Uncoupled NOS
- Superoxide → RNOS
- Redox-active Heme

Kidney

Arginase → ↑ Ornithine

Substrate Competition

↑ Polyamines

Vascular smooth muscle proliferation
Airway remodeling

↑ Proline

Collagen production/deposition
Lung fibrosis
Airway remodeling
Cannabis in the Treatment of Pain in Sickle Cell Disease

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ABSTRACT
Cannabis has been used for centuries for medical and recreational use across diverse cultures. This review highlights the experimental and clinical evidence to support the use of cannabis as medicine to treat pain in sickle cell disease (SCD). Current treatments for sickle pain are largely confined to opioids, thus limiting the treatment choices resulting in inadequate analgesia and significant adverse effects. This review highlights the experimental and clinical evidence to support investigating the use of cannabis as medicine to treat pain in sickle cell disease (SCD). The analgesic, anti-inflammatory and anti-oxidant activities of cannabis support investigating its use in the treatment of pain in SCD.

Introduction
Cannabis is one of the oldest known psychoactive plants.1,2 Contents of the tomb of a shaman in northern China dating back 2700 years include two receptacles containing flowers of the female Cannabis sativa plant, suggesting it was used for medicinal or divinational purposes.3 Medicinal cannabis likely followed the Silk Route to the Indian subcontinent and westward to the Arab world, where it was used for a variety of indications. Cannabis was introduced into western medicine when it was brought to the United Kingdom by W.B. O'Shaughnessy, a surgeon for the British East Indies Company who appreciated its analgesic, antispasmodic, anticonvulsant, anti-inflammatory and sedative properties. Most United States pharmaceutical companies produced cannabis medications in the early part of the twentieth century until it was removed from the nation's pharmacopoeia in 1942 following the passage of the Marihuana Tax Act. In 1970, cannabis was classified as a Schedule I drug with high potential for abuse and no known medical use in the Controlled Substances Act.

Cannabinoids and Their Receptors
Despite the ongoing prohibition against cannabis as medicine, much has been learned about how the plant's main components – the cannabinoids – affect the body. Two cannabinoid receptors have been identified.4-7 The CB1 receptor is primarily concentrated in the central nervous system, but is found diffusely in organs throughout the body. The CB2 receptor was initially identified in cells of the immune system – the spleen, B-lymphocytes and natural killer cells – suggesting it plays a role in immune function and inflammation. These receptors, members of the superfamily of seven-transmembrane spanning G protein-coupled receptors, are found in virtually all animal species. These receptors bind endogenous cannabinoids as well as the phytocannabinoids produced by Cannabis. As we produce endogenous opiates – the endorphins – we also produce endocannabinoids on demand from cell membrane lipids that complex with cannabinoid receptors and effect changes within target cells.8 Upon binding the CB1 receptor, endocannabinoid-induced signal transduction is thought to modulate pain, appetite, cognition, emesis, reward (addiction), neuroexcitability and thermoregulation.9 Through the CB2 receptor, endocannabinoids impact pain, inflammation, immune function and cell proliferation. Anandamide, named for the
Cannabis contains at least 100 twenty-one carbon terpenophenolic cannabinoid compounds. Delta-9-tetrahydrocannabinol (THC) is the main psychoactive component. Other phytocannabinoids, in addition to terpenoids and flavonoids, combine to produce an “entourage effect” – enhancing the beneficial effects of THC while reducing some adverse effects. For example, adverse effects of THC are reduced by coadministration with cannabidiol (CBD), a non-psychoactive cannabinoid with potent analgesic and anti-inflammatory effects. While most strains of Cannabis cultivated for recreational use are enriched for THC, medical consumers now seek high-CBD strains for analgesia without psychoactivity.

**Pain in Sickle Cell Disease**

Sickle cell disease (SCD) is characterized by vaso-occlusive crises (VOC) causing severe pain that may begin in infancy and continue throughout life. In a study spanning approximately 32,000 days, adult SCD patients experienced chronic pain on more than 54% of days. Pain from VOC is considered worse than labor pain and organ damage.

The mechanisms underlying pain in SCD remain poorly understood. The complex pathophysiology of SCD involves systemic inflammation, neurogenic inflammation, oxidative stress, vascular dysfunction, and end-organ damage. Hypoxia/reperfusion (HR) injury in VOC may aggravate these derangements, and in turn exacerbate pain. Transient receptor potential cation channel V1 (TRPV1) ion channels are activated in the peripheral nerve endings of sickle mice, which also show peripheral nerve damage. We found that neurogenic inflammation characterized by increased substance P (SP) and calcitonin gene-related peptide (CGRP), as well as increased inflammatory cytokines in the periphery and spinal cord, contribute to sickle pain. Mast cells play a key role in the activation of nociceptors by releasing SP, which activates protease activated receptor 2 (PAR2) and co-activates TRPV1 on peripheral nerve endings, leading to a vicious cycle of neurogenic inflammation and pain. Sustained chronic pain appears to be an outcome of central nociceptor sensitization in sickle mice accompanied by the constitutive activation of p38MAPK signaling, a key player in central sensitization. Increased circulating substance P in the blood of sickle patients as compared to normal subjects as well as emerging fMRI evidence of central sensitization further exemplify the complexity of sickle pain. The characteristic features of pain observed in sickle mice have been validated in human studies. Thus, pain in SCD appears to be inflammatory as well as neuropathic, and challenging to treat perhaps due to both peripheral and central sensitization.

**Cannabis and Analgesia**

**Endogenous and synthetic cannabinoids**

Controversies surrounding recreational drug usage and lack of evidence-based medical understanding of cannabis led to intense scientific efforts to enhance the production of endogenous cannabinoids. Endocannabinoids including anandamide have both anti-inflammatory and analgesic activity. However, the rapid metabolism of endocannabinoids limits their effectiveness. One of the key enzymes involved in this process is fatty acid amide hydrolase (FAAH). Several FAAH inhibitors have been developed that increase production of anandamide and other endogenous fatty acid amides such as N-arachidonyl glycerol (2AG). FAAH inhibitors significantly lower pain in experimental pre-clinical models. However, a placebo-controlled Phase II clinical trial with FAAH inhibitor PF-04457845 did not show a significant improvement in pain in patients with osteoarthritis of the knee. In this study, at least 4 endogenous substrates of FAAH were substantially increased, FAAH activity was reduced by >96% and no adverse events were reported.

Cannabis has been shown to be effective as a treatment in a rat model of neuropathic pain. The current therapy for neuropathic pain is generally inadequate. Opioids are often ineffective with high addiction potential in treatment of patients with chronic, non-life-threatening neuropathic conditions. In the past, patients with HIV infection frequently experienced painful peripheral neuropathy, caused either by the virus itself or some earlier antiretroviral therapies. Based on the preclinical model and anecdotal information from patients, Abrams et al conducted a pilot open-label study of inhaled cannabis in...
patients with HIV-related painful peripheral neuropathy, followed by a 50-patient randomized, placebo-controlled clinical trial.\textsuperscript{34} Participants were also subjected to a heat-capsaicin experimental pain model to serve as an objective anchor. Upon smoking the first study cigarette, the cannabis group experienced a 72% reduction in neuropathic pain compared to a 15% reduction in the placebo group. Over the ensuing 5-day study period, 52% of the cannabis group reached the threshold 30% reduction in chronic neuropathic pain, compared to only 24% of the placebo group. The area of secondary hyperalgesia in the experimental pain model was unchanged in the placebo group but declined significantly in cannabis recipients. The number of patients needing treatment for one to experience benefit (number needed to treat [NNT]) in our study was 3.6, which is comparable to the NNT for gabapentin in other peripheral neuropathic pain syndromes. In another Phase II placebo-controlled crossover dose-escalating study of cannabis in patients with HIV neuropathy, the NNT was 3.5, suggesting consistency and supporting the notion that cannabis is useful in HIV-related neuropathy.\textsuperscript{35}

Three subsequent studies investigated cannabis in other neuropathic pain syndromes. In 38 patients with neuropathic pain from complex regional pain syndrome, a linear analgesic dose response was seen in high and low dose cannabis groups but not with placebo.\textsuperscript{36} The investigators concluded that the analgesic effect was not anxiolytic, but that cannabis reduced both core nociception and the emotional response to pain. Twenty-three patients with post-traumatic and postsurgical neuropathic pain inhaled different doses of cannabis (including 0% THC) three times daily for five days.\textsuperscript{37} The average daily pain intensity was significantly lower on the highest THC strength (9.4%) with patients also reporting improved sleep quality. A sixteen patient Phase II double-blinded, placebo-controlled crossover study investigating different doses of cannabis (1%, 4% and 7% THC) in patients with diabetic neuropathy was recently completed.\textsuperscript{38} There was a significant difference in spontaneous pain scores between doses, with a dose-dependent reduction in patients with treatment-refractory pain.

A systematic review investigated published studies of cannabis-based medicinal extracts in different populations of patients with chronic nonmalignant neuropathic pain.\textsuperscript{39} Of 24 published studies, 11 were included in the review. The investigators concluded that cannabinoids may provide effective analgesia in chronic neuropathic pain conditions refractory to other treatments. They suggest further high-quality studies are needed to assess the impact of treatment duration and optimal drug delivery modalities. Additional systematic reviews of cannabis in non-neuropathic chronic pain syndromes also have concluded that cannabinoids are safe and effective analgesics and provide a reasonable therapeutic option.\textsuperscript{40,41}

Cannabinoids and opioids share several pharmacologic properties including antinociception, sedation, hypothermia, hypotension and inhibition of intestinal motility. Cannabinoid receptors do not risk respiratory depression because cannabinoid receptors, unlike opioid receptors, are not concentrated in the brainstem. Cannabinoids interact with kappa and delta receptors in production of analgesia. Opioids achieve analgesic effects through mu receptors, which may be enhanced by cannabinoids. In mice and rats, THC synergistically augments the analgesic effects of morphine.\textsuperscript{42–44} Supplementing lower opioid doses with cannabinoids may enhance and sustain analgesia if such an interaction occurred in humans.

To investigate potential cannabinoid:opioid interaction, we conducted a classical pharmacokinetic interaction study involving twenty-one patients with chronic pain on a stable dose of sustained release morphine and eleven patients on sustained release oxycodone. The causes of pain in these patients included unspecified musculoskeletal pain (n=7), post-traumatic (4), arthritis (2), peripheral neuropathy (2), as well as cancer, fibromyalgia, migraine, multiple sclerosis, sickle cell disease and thoracic outlet syndrome (n=1 each).\textsuperscript{44} An initial 12-hour opioid area concentration versus time curve was obtained at baseline. Participants then inhaled vaporized cannabis three times daily for four days with repeat opioid kinetics drawn on day five. Despite no change in the oxycodone concentration curves and a mild decrease in the plasma levels of morphine after cannabis exposure, patients reported a significant 27% overall decrease in chronic pain with combination therapy, suggesting a possible synergistic pharmacodynamic – not pharmacokinetic – effect. Continuous oxygen saturation monitoring was done throughout the trial. No adverse effects were seen in any patients, including one participant who had sickle cell disease. This provided us with some basis to suggest it would be safe to conduct our current study in patients with sickle cell disease.

Nabiximols (Sativex®) is a whole cannabis extract oromucosal spray with a standardized THC:CBD ratio that is available in Canada and European nations.\textsuperscript{45} Originally approved for treatment of pain and spasticity associated with multiple sclerosis, nabiximols is being evaluated in an ongoing Phase 3 trial in the US for patients with cancer-associated pain. It may provide a non-inhaled option for cannabis-based medicine in other pain conditions as well.

**Cannabis in Sickle Cell Disease**

**Animal Studies**

**Cannabis to treat sickle pain**

Preclinical studies suggest that cannabis may ameliorate pain and address the underlying pathophysiologic changes in SCD (Table 2). We found that CP55,940, a high affinity
CB1 receptor (CB1R) and CB2 receptor (CB2R) agonist, significantly reduced chronic and hypoxia-reoxygenation (HR)-evoked pain in HbSS-BERK sickle mice. These mice closely recapitulate clinical and pathophysiological features of SCD including chronic pain, neurogenic inflammation, endothelial activation, reticulocytosis, end-organ damage, and shorter lifespan. CP55,940 also ameliorated features of sickle pain including increased sensitivity to touch and temperature extremes, musculoskeletal/deep tissue pain, and HR-evoked pain in sickle mice.

Interestingly, the CB1R agonist ACEA and CB2R agonist JWH-133 both attenuated deep hyperalgesia, but only ACEA reduced HR-evoked mechanical and thermal hyperalgesia. While CB1R is more critical for analgesia, CBD and the CB2R pathway have been demonstrated to ameliorate pain at least in part via TRPV1 and at the supraspinal level in animal models of neuropathic pain. Pain is also accompanied by stress in SCD. Stress-induced neuroinflammation was significantly attenuated in wild type mice treated with JWH-133 and mice overexpressing CB2R, but not in CB2R-knockout mice. Therefore, CB2R agonists augment CB1R analgesia in sickle pain, and both may be required to achieve effects similar to those from whole plant based cannabis.

Cannabinoids attenuate inflammation, leukocyte trafficking and adhesion, mast cell activation, oxidative stress, ischemia reperfusion (IR) injury and neurogenic inflammation via CB1R as well as CB2R (Table 2). All these phenomena exacerbate pain and may underlie clinical features of SCD including impaired wound healing, renal damage, and retinopathy. Cannabinoid receptors are expressed on the endothelium, inflammatory cells, central nervous system (CNS) and most bodily tissues. Cannabinoids may therefore target multiple features of sickle pathology as described below.

Inflammation: Severe inflammation in SCD is characterized by elevated cytokines ("cytokine storm"), pro-inflammatory and vasoactive neuropeptides, in both humans and sickle mice. Microglial activation with significantly higher cytokine levels, TLR4 expression and Stat3 phosphorylation in sickle mice spinal cords suggest a central inflammatory milieu. In animal models of diverse diseases, CB2R was found to mediate the anti-inflammatory effect of cannabinoids such as CBD, HU210, and WIN 55,212-2, both peripherally and centrally. THC via CB1R also has an anti-inflammatory effect, but CB2R appears to play a critical role in regulating inflammation in most cellular and animal studies.

Neurogenic inflammation: Sickle mice also show pronounced peripheral neurogenic inflammation. We observed that mast cell activation contributes to neurogenic inflammation in sickle mice. Cannabinoids inhibit mast cell degranulation via CB2R as well as CB1R. Leukocyte rolling and adhesion also play critical roles in sickle pathobiology, and along with red blood cell (RBC) adhesion is critical in the process of painful VOC in SCD. In a kaolin-carragenan-induced inflammation model in C57BL/6 mice, URB597 (a monoacylglycerol lipase inhibitor) decreased leukocyte rolling and vascular permeability via both CB1R and CB2R, while analgesia was mediated via CB1R and leukocyte adhesion was inhibited in a CB1R-independent manner. Thus, both CB2Rs may be involved and synergize with other non-CBRs to reduce hyperalgesia in the complex peripheral and central sickle milieu.

Ischemia/reperfusion (IR) injury: IR injury is a hallmark feature of SCD and evokes pain. Cannabinoids reduce IR injury via both CB1R and CB2R, although CB2Rs appear to play a major role in most preclinical studies involving different pathological conditions. JWH-133 protected myocardial IR injury in a rat model by inhibiting myocardial apoptosis via PI3/Akt signaling. We found that JWH-133 significantly attenuated HR-evoked mast cell activation and reduced serum SP levels in sickle mice, while ACEA led to non-statistically significant reduction, suggestive of a CB2R-mediated response. However, attenuation of HR-evoked neurogenic inflammation and pain were dependent upon both CB1R and CB2R. Similarly, in neuropathic pain models using CB1R and CB2R knockout mice, both CB1R and CB2R were involved in ameliorating pain by 2-AG, URB602 and WIN55,212-2.

Oxidative Stress: Inflammation, hemolysis, and cell-free hemoglobin in the hypoxic sickle microenvironment cause oxidative stress in SCD. WIN55,212-2, CP55,940 and anandamide exert a protective effect on quinolinic acid-induced mitochondrial dysfunction, reactive oxygen species (ROS) formation and lipid peroxidation in rat striated cultured cells and rat brain synaptosomes. Importantly, in parkin-null, human tau overexpressing (PK−/−/TauV LW) mice, a model of complex neurodegenerative disease, short-term Sativex administration significantly reduced intraneuronal monoamine oxidase (MAO) related free radicals, increased the ratio of reduced vs oxidized glutathione (GSH), and improved behavioral and pathological abnormality. Consistent with these observations in other pathologies, cannabinoids may also reduce oxidative stress and pain in SCD.

Endothelial activation: Erythrocyte adhesion, nitric oxide depletion, hemolysis, oxidative stress and inflammation accompany endothelial dysfunction in SCD. Endothelial activation causes upregulation of adhesion molecules including selectins, vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM), which
exacerbate vaso-occlusion and end-organ damage. CB1R and CB2R are widely expressed on vascular smooth muscle cells and endothelium. Both receptors have been widely studied in vascular relaxation and activation of ion channels including potassium, calcium and TRPVs. Antagonistic roles are demonstrated in different settings and disease states with respect to CB1R and/or CB2R. Thus, it will be critical to examine the endothelial effect of cannabinoids in a sickle-specific microenvironment.

Human Studies

There is a paucity of literature on the use or effects of cannabis in patients with sickle cell disease. Two small studies of usage have been published. Investigators evaluated the prevalence of cannabis smoking in the Jamaica Sickle Cell Cohort Study by asking participants about use in 2000 and again in 2004. Cannabis smokers were asked whether they used it for complications of sickle cell disease. Patients with both SS and SC disease were included. The mean age of participants was 22.6 years in 2000 and 26.6 years in 2004. There were 175 SS patients and 113 SC patients responding in 2000, and 149 SS patients and 85 SC patients in 2004. In 2000, cannabis smoking was reported by 49% of men with SC disease and 32% of men with SS disease, but only 6% and 4% of women, respectively. Estimates of cannabis use by the general Jamaican adult population range from 37-49% for men and 10-15% for women. By 2004, 49% of men with SC and 62% of men with SS endorsed cannabis smoking, with usage increasing to 29% and 19% of women, respectively. Of those who reported cannabis use in 2004 and attended both reviews, 97% began smoking between 2000 and 2004. Only 11% in the 2004 survey reported that they used cannabis because of complications of sickle cell disease. Seven patients said they used cannabis for painful crises while others identified depression, asthma or weight loss as motivating reasons. Pain crises were counted and the investigators found no suggestion of different pain patterns between cannabis smokers and non-smokers. The odds of smoking did not increase with increasing pain in either 2000 or 2004. The authors conclude that despite a significant proportion of the Jamaican sickle cell population having smoked cannabis, most usage has not been specifically for sickle cell morbidity.

A questionnaire was offered to adult patients with sickle cell disease at the Central Middlesex Hospital in London prompted by investigators receiving anecdotal evidence of cannabis being used for analgesia from confidential accounts. Eighty-six questionnaires were completed over a 6-month period. Thirty-one patients (36%) reported using cannabis, the majority by inhalation with only 3 reporting oral ingestion. The frequency of use was daily (4 patients), weekly (10 patients), monthly (4 patients) with the rest reporting occasional use. Sixteen people used cannabis for medicinal reasons, mainly to reduce acute or chronic pain and to decrease other analgesic intake. There was no evidence of more severe sickle cell disease in these patients except that avascular necrosis of the femoral head was more common than in the 15 recreational users (4 cases versus 1). Eleven of 13 people who listed sleepiness as a side effect found it beneficial. The authors suggest that cannabis may be useful for relief of acute and chronic pain and decreasing opioid analgesic use.

Proof of Principle Study

Chronic pain causes significant morbidity in SCD. Many patients continue experiencing chronic pain and episodic acute crises despite opioid maintenance. An increasing number of states across the United States have established provisions for patients to utilize cannabis for medicinal purposes. It is likely that significant numbers of patients with various medical conditions utilizing opioid analgesics might self-medicate with inhaled cannabis to augment analgesic effects. No clinical information exists on the potential effectiveness of adjunctive cannabis in reducing chronic pain, decreasing opioid use, decreasing vaso-occlusive crises or decreasing utilization of medical care in SCD patients. Building upon the preclinical studies discussed above, we are now conducting a human proof of principle trial.

Cannabidiol (CBD), a non-psychoactive cannabinoid, is felt to have potent anti-inflammatory and analgesic activities. CBD has low affinity for the CB1 and CB2 receptor, and may interact with the endocannabinoid system as an FAAH inhibitor. Cannabidiol exerts multiple central and peripheral analgesic, anti-inflammatory, antioxidant, and neuroprotective effects. CBD may be useful in SCD because of its previously observed utility in treatment of pain, neurodegenerative diseases and ischemia. To date, cannabis containing significant amounts of CBD has not been utilized in clinical investigations in the United States. Hypothesizing that this anti-inflammatory analgesic could be beneficial in patients with chronic sickle cell pain, we elected to study a cannabis preparation with a THC:CBD ratio of approximately 1:1.

This randomized placebo-controlled crossover study is enrolling 35 adults with sickle cell disease and measurable chronic pain, on or off maintenance opioid analgesics. The study is comprised of two 5-day intervention periods in the San Francisco General Hospital inpatient clinical research center. Participants complete a 5-day daily pain diary prior to admission to establish an outpatient pain baseline. On Day 1 of admission, subjects provide blood for baseline markers of inflammation and SCD progression that will be analyzed at the University of Minnesota laboratory. Pain is assessed using the Brief Pain Inventory and a visual analogue scale. At 12pm on Day 1, participants inhale their
first vaporized cannabis dose. Vaporization has been shown to be an effective smokeless delivery system that results in comparable blood cannabinoid levels and physiologic effects to inhaling a cannabis cigarette. 71 Participants inhale either mixed THC/CBD or placebo three times daily on Days 2 through 4. The pain visual analogue scale is completed daily throughout the study. On Day 5, subjects vaporize for the final time at 8am and complete the Brief Pain Inventory again. Subjects continue taking their pre-study analgesic medications (e.g., opioids, gabapentin, amitriptyline, nonsteroidal anti-inflammatory drugs [NSAIDs]) at a stable dose while enrolled. If additional analgesia is required during the inpatient study period, supplemental therapy is given and the dose recorded. On Day 5, repeat specimens for markers of inflammation and SCD progression are obtained.

Precautions in Patients with Sickle Cell Disease

The pulmonary risks of inhaling cannabis as combusted plant material have been well-investigated. 72-74 Acute and chronic bronchitis seem to be the most commonly recognized complications. There is little evidence that inhalation of cannabis without tobacco increases the risk of chronic obstructive pulmonary disease or pulmonary neoplasms. Any risks associated with smoking cannabis are likely diminished with vaporization. The impact on the induction of vaso-occlusive chest syndrome in patients with SCD is unknown. The epidemiologic studies from Jamaica and London do not suggest an increase in painful crises in cannabis users with SS or hemoglobin S-C disease.

Physiologic effects of cannabis including lower blood pressure and heart rate may be undesirable in SCD patients with high-output heart failure or occlusive coronary artery disease. 75 A 15-year longitudinal study failed to identify any significant risks of long-term cardiac consequences of cannabis use in young adults. 76

A literature search also reveals a case report of a patient with sickle trait developing prolonged priapism after exposure to cannabis. 77 Whether this finding is uniquely associated with SC trait is unclear.

Conclusion

To date, research with cannabis has not focused on its potential therapeutic value. As a controlled Schedule I substance with no known medical use and a high potential for abuse, cannabis is difficult to obtain for clinical trials. Conducting a trial requires funding agency (often the National Institutes of Health) approval, National Institute on Drug Abuse-supplied cannabis, a Food and Drug Administration investigational new drug (IND) application, a federal Drug Enforcement Agency (DEA) Schedule I license and local DEA approval of the drug storage facility. Additional state and local reviews must also occur.

As more states are approving cannabis for medicinal use, there is a demand from physicians and other health care providers for evidence of its effectiveness from controlled clinical trials, which as yet is non-existent. Even if evidence is generated, physicians practicing medicine today were raised during an era of cannabis prohibition and even demonization, and have no significant experience discussing its medicinal use with patients. During an annual meeting of the American Association of Pain Management in 2012, the audience was polled about medicinal cannabis. The prompt was “I recommend that my pain patients try cannabis,” with options of “never”, “rarely”, “sometimes”, “often”, or “usually.” Among the 72 respondents, 35% chose never, 24% rarely, 29% sometimes, 7% often and 6% usually, reflecting very limited use of this potentially useful analgesic. In contrast, a vast majority of patients surveyed (83-97%) utilizing medicinal cannabis in California and Hawaii reported using it for chronic pain. 78, 79 Hopefully, increased continuing medical education offerings on therapeutic cannabis use will ameliorate this situation and promote a re-branding of cannabis particularly for chronic pain. 80

The potential for cannabinoids to decrease use, abuse and adverse effects of opioids is most attractive. An analysis of cannabis use among 1514 people using opioids for chronic pain in Australia reported greater pain relief with cannabis than with opioids used alone. 81 Evidence is emerging that in states making medicinal cannabis available, deaths attributed to opioid use have declined. 82 Patients with SCD often become tolerant to opioid therapy. If cannabis proves to be a valuable adjunct to available analgesics, it could have a significant impact on overall health and quality of life in this patient population.

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Contribution: MG co-wrote the manuscript and prepared for submission; ST researched the literature and prepared the Tables; KG wrote the sickle and experimental components and edited the manuscript; DIA wrote the human studies and edited the manuscript.
REFERENCES


62. Desroches J, Harron S, Bouchard JF, Beaulieu P. Endocannabinoids decrease neuropathic pain-related behavior in mice through the activation of one or both peripheral CB(1) and CB(2) receptors. *Neuropharmacology*. 2014;77:441-452.


References:


ABSTRACT

This review describes current clinical considerations in the use of hydroxyurea for the management of sickle cell disease (SCD). Although the genomic influences on fetal hemoglobin (HbF) levels in sickle cell disease are currently being elucidated, and although the efficacy hydroxyurea is mediated primarily through increased HbF levels, our understanding of hydroxyurea’s pharmacokinetics and pharmacogenomics remains limited. This review summarizes the history of clinical trials leading to the current rationale for hydroxyurea therapy in SCD, including its beneficial effects on mortality, and focuses on recent information gained from trials that include evaluation of its effects on organ function. Although some benefit in brain, splenic, renal, pulmonary, and retinal function have been reported, the effects of hydroxyurea on organ function have been considerably less striking than those seen in the reduction of acute vasoocclusive events. Current information regarding the drug’s safety and toxicity, and current dosing regimens are also described. The recommendations of the 2014 NIH Evidence-Based Management of Sickle Cell Disease Expert Panel Report conclude this review.

Introduction

Hydroxyurea (HU) is currently the only drug approved by the U.S. Food and Drug Administration (FDA) for treatment of sickle cell disease (SCD) and is the most common intervention utilized in its long-term management. Recent articles have reviewed the mechanisms of action of HU and provided a comprehensive update of the clinical effects of this drug (1, 2). This review will summarize the status of the use of HU for SCD in 2015, with particular emphasis on current information regarding its effects on fetal hemoglobin and on organ function.

Fetal Hemoglobin (HbF)

Fetal hemoglobin (HbF) inhibits the polymerization of deoxyhemoglobin S because neither HbF (αγζ) nor the hybrid tetramer αγβζ is incorporated into the polymer (3). The effect of HbF in ameliorating the clinical and hematologic complications of sickle cell disease has been recognized for decades. Early observations included the delayed onset of manifestations of sickle cell disease during the first 4-6 months of life before fetal hemoglobin declines to low levels, the protection offered by inheritance of a hereditary persistence of fetal hemoglobin (HPFH) gene in combination with that for HbS, the relatively mild symptomatology of subjects from India and eastern Saudi Arabia with high fetal hemoglobin levels, and numerous observations from the Cooperative Study of Sickle Cell Disease (CSSCD) in which higher HbF was associated with diminished rates of pain events, acute chest syndrome, and mortality (4). After stabilization of HbF levels at about age 4 years, baseline values in persons with sickle cell anemia (SCA) average 8-10% (range ~3-20%).

Studies in siblings with SCD have shown a high heritability of HbF levels (5). Regulation of the expression of HbF is known to operate both within and external to the region on chromosome 11 which contains the γ, δ, and β globin loci. At least three genetic loci influence HbF levels and account for >20% of the variability of those levels in SCD patients (6). BCL11A is an oncogene on chromosome 2 (2p16) that binds to the β-globin locus and promotes the switch from γ to β-globin, dampening expression of HbF. Second, polymorphisms in the HBS1L-MYB (HMIP) intergenic region on chromosome 6 are associated with F-cell levels. Third, polymorphisms in the Xmn1 site upstream of the γ-globin locus on chromosome 11 (HBG2) are associated with higher HbF levels and milder disease.

Hydroxyurea — Mechanisms of Action

5-azacytidine was the first DNA modifying agent shown to increase HbF (in anemic baboons) (7). Initial trials of 5-azacytidine in adults with sickle cell anemia showed increases in HbF production and reduced dense cells (8), but human studies were discontinued after rat models showed potential for increased tumorogenicity (9). In 1984 attention switched to HU following reports that both anemic monkeys and sickle cell patients had increased HbF production when treated with HU (10, 11) (Table 1). Hydroxyurea is a ribonucleotide reductase inhibitor that has been used to treat myeloproliferative disorders and chronic myelogenous leukemia since the 1960s (12). However, the exact mechanisms by which HU induces HbF production are not fully understood. Cytotoxic effects of HU on the bone marrow produce stress erythropoiesis with increased HbF production (13). In vitro studies utilizing K562 erythroleukemia cells and human erythroid progenitor cells have demonstrated that HU nitrosylates and activates...
soluble guanylate cyclase (SGC) with subsequent increased expression of cyclic GMP (cGMP)-dependent protein kinase (14, 15). The nitric oxide (NO)-SGC-cGMP pathways are thought to play a role in induced expression of γ-globin.

Hydroxyurea has a number of beneficial effects besides induction of HbF, including decreased neutrophil and reticulocyte counts caused by narrow cytotoxicity, reduced expression of adhesion molecules on neutrophils and red cells, increased erythrocyte hydration and deformability with subsequent reduction in hemolysis, and local release of nitric oxide (NO), potentially resulting in vasodilatation (16, 17, 18). Another mechanism was suggested from a study of HU in sickle cell mice, in which their survival from pneumococcal pneumonia was improved by reduction of their abnormally elevated inflammatory response (19). A reduction in adenosine activity in HU-treated patients whose monocytes express CD26 may also be beneficial (20).

**Hydroxyurea – Clinical Trials**

Only four randomized placebo-controlled trials of the use of hydroxyurea in sickle cell anemia (SCA) have been published (21-24). Following a phase I-II trial (25), a landmark double-blinded phase III trial which led to FDA approval of the drug for adults with clinically severe SCA was the Multi-Center Study of Hydroxyurea (MSH), in which 298 patients were randomized to receive HU or placebo (21). The study was halted early due to statistically significant increase in the time to the first painful vasoocclusive event in the HU arm. Persons randomized to this arm had 40-50% reductions in the incidence of painful events, acute chest syndrome, transfusions and hospitalizations. The first randomized trial in children was a single-blind crossover study in which patients received six months of HU and six months of placebo. The 22 evaluable patients demonstrated reduction in the number of days in the hospital and the length of hospital stay when receiving HU (22). The largest of several non-randomized studies in children with SCA in the U.S. was HUG-KIDS, a phase I-II trial in 84 school age children with sickle cell anemia (26), which showed similar hematologic responses to those seen in adults, including increases in HbF, MCV, and hemoglobin levels and decreases in WBC and absolute neutrophil counts. More recently, a phase I-II study (27) served as a pilot for the phase III double-blinded multi-center BABY HUG trial (23) which involved 193 infants between 9 and 18 months of age who were randomized to receive hydroxyurea at a fixed dose of 20 mg/kg/day or placebo for a two-year period, with the primary objective of demonstrating better preservation of organ function in the spleen (based on uptake on Tc⁹⁹ liver-spleen scans) and kidneys (based on glomerular filtration rate measured by Tc⁹⁹DTPA renal clearance). Although the primary endpoints were not achieved, the study demonstrated that the placebo group had fewer episodes of pain, dactylitis, acute chest syndrome, hospitalizations and transfusions, along with higher levels of hemoglobin, HbF, and MCV, and decreased WBC, neutrophil, and reticulocyte counts. Administering daily hydroxyurea in a liquid formulation was feasible and well tolerated with no significant toxicity, except for the expected mild to moderate neutropenia, which was not associated with increased risk for invasive infection. Most patients from the BABY HUG trial continue in a follow-up study, which will be crucial for identifying long-term benefits and risks. In a fourth randomized trial involving sickle cell children from India, patients who were randomized to a low fixed dose of HU had increased Hb and HbF levels and fewer pain episodes, transfusions and hospitalizations compared to those on placebo (24).

Hydroxyurea has a beneficial effect on mortality (Table 2). In a 17.5 year follow-up of the MSH study, mortality was significantly reduced in individuals with long-term HU exposure (28). In the Greek LaSHS study of 330 adult patients with HbSS, Sβ⁰- or Sβ⁻-thalassemia, significant reductions in pain, acute chest syndrome, transfusion, hospitalization, stroke and overall mortality occurred (10-year survival in the HU-treated group 86% versus 65% in those untreated) (29). In a report from Rio de Janiero, Brazil, among 1760 children with sickle cell disease, 267 were treated with HU because of clinical severity for a median of 2 years (30). Although this was not a randomized trial, the HU-treated children had significant reductions in hospitalizations, transfusions, and, most notably, mortality, due to fewer deaths from acute chest syndrome and infection (99.5% vs. 94.5% survival, p=0.01).

**Hydroxyurea and Organ Function (Table 3)**

The efficacy of HU in the reduction of vasoocclusive events (pain, acute chest syndrome) has been established through randomized, placebo-controlled trials (MSH, BABY HUG) and numerous observational studies in adults and children (21, 23). However, clear understanding of its value in the prevention, stabilization, or reversal of organ dysfunction in patients with sickle cell disease remains an elusive goal. In Table 3 we have summarized recent studies of organ function in patients receiving HU, although the data are often limited and inconclusive or contradictory.

Because both splenic and renal function are affected very early in life in patients with SCA, these two organs have been the focus of a number of studies. The first primary endpoint of the BABY HUG trial was a comparison of splenic function at study exit (after 2 years of HU or placebo) with uptake at entry (23). Splenic uptake
on scan was classified as normal, decreased, or absent. Using these criteria, 27% of patients on HU had a decrease in spleen function compared with 38% in those receiving placebo; this difference was not statistically significant. However, secondary measures, including quantitation of pitted red blood cells and Howell-Jolly bodies, showed significantly greater increases in these markers of absent splenic function in those receiving placebo compared to those on HU (31). Several observational reports of spleen function have involved small populations of sickle cell patients, mostly school-age children at single institutions. For example, spleen scans in 40 children with a mean age of 9.1 years demonstrated at least some splenic uptake in one third after three years of HU treatment (32).

Renal function assessed by glomerular filtration rate (GFR) measured by $^{99m}$TcDTPA clearance was the second primary endpoint of the BABY HUG study; no significant lessening of the elevated GFR in the HU group was found (23). However, secondary endpoints, including urine osmolality and urine specific gravity after overnight fasting, were higher in HU-treated patients and total kidney volume measured by ultrasound scanning was greater in the placebo group (33). HU may have a beneficial effect on microalbuminuria/proteinuria. Proteinuria was lessened in three school-age patients with the combination of HU and enalapril; other observational studies in children found diminished microalbuminuria on HU (34, 35). In a recent report involving school-age children with SCA, HU treatment did not reduce microalbuminuria but was associated with decreased GFR (36).

Recent exploration of the effects of HU on the central nervous system (CNS) has included randomized trials that examined both primary and secondary stroke prevention in comparison with chronic transfusion (Table 3). Standard management for primary stroke prophylaxis has been based on the landmark STOP trial, which demonstrated that chronic transfusion in children with abnormally elevated transcranial Doppler ultrasound (TCD) velocities in the internal carotid and middle cerebral arteries was 90% effective in the prevention of initial strokes (37). Consistent with these results, in a Parisian study of 54 young children with SCA and normal TCD and MRA, only two developed an abnormal TCD during 225 total patient years of observation, a very low rate (38). In addition, several reports in children with SCA, some of whom had velocities in the abnormal range but were not candidates for chronic transfusion, described decreased TCD velocities after starting HU, indicating a reduced risk for stroke (39-42). These findings led to the multi-center randomized TWITCH trial in which subjects with abnormal TCD velocities who had received chronic transfusion for at least a year and who did not have severe stenosis on MRA were randomized to continue chronic transfusion or substitute HU for chronic transfusion (43). This trial has recently been closed because the endpoint of “non-inferiority” of the HU group had been reached, but publication of the final results is pending. At the conclusion of the BABY HUG study, there was no significant difference in the mean TCD velocities of the HU and placebo arms, but the age-related increase in velocity between entry and exit was significantly greater in the placebo arm (23). Currently, an ongoing trial in Nigeria is examining the use of HU as the initial intervention for stroke prevention in patients with abnormal TCDs because chronic transfusion cannot be safely administered there (SPIN Trial, NCT01801423) (44).

The role of HU in secondary stroke prophylaxis has also been examined in a multi-center randomized trial (SWITCH) (45). Standard management for sickle cell patients who have suffered an overt ischemic stroke has been chronic transfusion, despite its attendant toxicities of iron overload and alloantibody induction. This intervention is effective in reducing the stroke recurrence rate from approximately 60-70% to 15%, but transfusion must be continued indefinitely. In the SWITCH trial, standard management with continued chronic transfusion (plus iron chelation) was compared with HU treatment (plus phlebotomy for reduction of iron overload), but the study was halted after the occurrence of 7 strokes in the HU group (versus none in the chronic transfusion group) in conjunction with a lack of superior efficacy from phlebotomy for iron reduction. Several other studies have evaluated smaller cohorts of patients who were receiving HU for secondary stroke prevention, but results were mixed (46-49). A recent study from Jamaica found that HU was more efficacious for secondary stroke prevention than no treatment at all (50). Thus, HU cannot be recommended for secondary stroke prevention if safe chronic transfusion is available.

The role of HU in the prevention or management of silent cerebral infarcts, which may occur in up to 38% of children with HbSS, is unclear at this time. In the recent Silent Cerebral Infarct trial (SIT), chronic transfusion reduced the frequency of new overt/silent infarcts in children with SCA and SCI on MRI screening compared to observation alone, but patients receiving HU had been excluded from the study at entry (51).

The effects of HU on psychometric performance in children with SCD are even less certain. In a small study comparing 15 children with SCD on HU with 50 not on HU, the former had better verbal comprehension and general cognition (52). In the BABY HUG trial, infants with SCA who were randomized to receive HU for two years had no difference in the Bayley mental developmental index (MDI) score at study exit compared with those receiving placebo, but 5 children in the placebo group had an MDI below 70 compared with none in the HU group (23).
The effects of HU on cardiopulmonary function have been examined in several observational studies. In a review of 10 studies that examined pulmonary hypertension based on tricuspid valve regurgitant (TR) jet velocity, HU effects were inconsistent (54). For example, in a study involving 152 children on HU, there was no difference in TR jet velocity compared to 247 children who were not receiving the drug (54). In the two studies in which pulmonary hypertension was measured by right heart catheterization, no effect from HU was seen (55, 56). In the MSH follow-up study, after 17.5 years, there was 24% mortality due to pulmonary causes and 87% of these patients had received HU for only five years or less (28). In the LaSHS follow-up study, HU was not associated with decreased mortality from pulmonary hypertension (29). Although the above evidence suggests that HU is not beneficial for pulmonary hypertension in SCD, a study of 41 male school children from Oman, found that exercise tolerance was increased on HU (57).

Growth in childhood was not adversely affected by HU and, if anything, may have benefitted in two multicenter trials: 5-16 year olds in HUG-KIDS (58) and 9-18 month olds in BABY HUG (59). Among studies of other organs affected by SCD, four of five adult men with recurrent priapism benefited from HU (60). Recently, children with HbSS were found to have a 7 fold greater likelihood of developing nephropathy if their HbF was less than 15% and HU-related HbF induction was likely beneficial (61).

Toxicity and Safety

The long-term safety of HU remains to be fully determined, although patient populations have now been followed for 15-20 years (28, 62). The primary short-term toxicity has been dose dependent, transient and reversible myelosuppression affecting the absolute neutrophil count; anemia/reticulocytopenia or thrombocytopenia are occasionally found. Melanonychia and skin hyperpigmentation have been the only physical findings clearly attributable to HU and occur in a small proportion of cases (63). GI complications, such as nausea, vomiting and diarrhea, have not occurred more frequently in the HU-treated population when compared with controls.

Hydroxyurea in high doses acts as a mutagen, teratogen and carcinogen in animals and in vitro (64-66). In a study utilizing RBC micronuclei as a marker of genotoxicity, increased micronuclei production was associated with substantial interpatient variability (67). In another study, chromosome damage was less for children receiving HU than untreated patients and there were no differences in repairing chromosome breaks after radiation (68). Data from the BABY HUG trial using chromosomal karyotype, VDJ recombination events, and micronuclei in reticulocytes as measures of genotoxicity, there was no difference between HU and placebo subjects (69). Although 6 cases of leukemia, mostly AML, have been reported in HU-treated subjects with SCD, the data do not suggest an increased risk of malignancy because of the large (but unknown) number of sickle cell patients who have received the drug. A recent review of health insurance claims in the U.S. found an adjusted AML risk ratio with HU exposure in adults with SCD of 0.94 compared to unexposed patients and concluded that there was no increased risk for AML (70).

A recent review of reproductive issues in SCD noted the impact of long-term therapies, including transfusional iron overload, hematopoietic stem cell transplantation, and possibly HU on fertility, and advocated more research on teratogenic effects of HU before abandoning its use during pregnancy (73). In an analysis of 94 pregnancy outcomes involving subjects who participated in the MSH trial, exposure of the fetus to HU did not cause teratogenic changes in those pregnancies that terminated in live births, whether the parent who took HU was the mother or the father (74). In another recent review, it was noted that “the vast majority of young men with SCA will have abnormal sperm analysis” (75). However, the evidence that HU results in further decline in concentration and function of spermatozoa is limited to case reports and a small series, precluding “strong conclusions” and indicating the need for investigation into the “reproductive epidemiology” of HU therapy (75, 76). Currently a prospective study in Paris is evaluating the effect of HU on spermatogenesis (NCT01609192).

Pharmacokinetics (PK)

Currently in the U.S., HU is approved by the FDA only in capsular form for adults with “severe” SCA. A liquid formulation with HU dissolved in simple syrup at a concentration of 100 mg/mL is commonly used in younger children (77), but FDA review is still pending.

PK evaluation of HU has been limited. In a French study, PK profiles in 11 children given a breakable HU tablet demonstrated a Cmax of 24.5 ug/mL and a Tmax of 0.75 hours (78). In another study, PK testing following a first dose of liquid HU showed a Cmax of 25.4 ug/mL and a T1/2 of 1.6 hours; both slow and fast concentration-time profiles were seen (79). Very recently, a solution of HU in cherry syrup compared with Droxia capsules showed bioequivalence in children, indicating that dosing...
adjustments for differences in either drug formulation were unnecessary (80). Although little is known about mechanisms that produce variability in HU pharmacokinetics, organic anion transporting polypeptide 1b (OATP1b) transporters have recently been shown to modulate HU PK (81).

Pharmacogenetics and Prognostic Factors

In the HUG-KIDS HU safety trial, the strongest predictor of HbF at maximum tolerated dose (MTD) was the baseline HbF level (82); a similar result was found in a more recent study, in which pharmacogenetic SNPs did not influence HbF% at MTD (79). In another study, baseline HbF explained 33% of the induced level of HbF, but a variant in the θ-globin locus, which added an additional 13%, was the only other contributor to the variance (83). In other recent studies, the Bantu haplotype conferred a greater response to HU treatment (84), a homozygous mutant state of a KLF10 SNP was associated with a poor response to HU (85), and a coding variant in SALL2 (Spalt-like transcription factor), identified through whole exome sequencing, was associated with a higher final HbF (86). In an ancillary analysis from the BABY HUG trial, alpha thalassemia, beta-globin haplotype, and polymorphisms affecting HbF levels (Xmn1, BCL11A, and HBS1L-MYB) were studied in 190 randomized subjects (87). At study entry, infants with alpha thalassemia trait had lower MCV, bilirubin and reticulocyte counts; beta-globin haplotypes were associated with higher Hb and HbF levels; and BCL11A and Xmn1 polymorphisms affected baseline HbF. At study exit, subjects randomized to placebo had the same associations with laboratory findings, but those receiving HU had drug treatment effects that superceded most genetic influences. Thus, in general, pharmacogenetic predictors of baseline HbF level, but not of HbF response to HU have been identified. Genetic studies of larger populations on HU and of unusual responders, and the influence of specific sequence variants, ideally analyzed with whole genome sequencing, are needed to better predict HbF response (1).

Dosing

"Standard" HU treatment is usually initiated with a dose of 20 mg/kg given once daily by mouth. However, controversy exists about the benefits and risks of gradually escalating the dose to MTD vs. maintenance at a fixed dose. In a 2010 review article, it was noted that hematologists in Europe tended to utilize a fixed dose, while those in North America typically escalated dosing to MTD (16). The review also noted that escalation to >25 mg/kg/d achieved laboratory thresholds of Hb >9 g/dL, MCV >100 fl, and HbF ~20%, all greater than the levels reached on a fixed dose of 20 mg/kg/d. However, no randomized trial has compared these two dosing strategies with regard to efficacy in reducing clinical symptoms and maximizing HbF levels vs. the likely concerns of increased hematologic toxicity and greater monitoring demands (and cost) of escalated dosing. Several recent reports even have suggested that fixed low dosing (10-15 mg/kg/d) might yield similar efficacy as higher dosing coupled with the advantage of less frequent monitoring requirements (88, 89). A multi-center trial is needed to resolve this question.

Combination Treatments

An area that has been mostly unexplored is the use of combination pharmacotherapy for SCD in which a well-studied effective drug (HU) might be combined with a relatively experimental and/or underutilized agent (90). Ideally, the agents used in combination should have different mechanisms of action, non-overlapping and relatively limited toxicities, ease of administration, and non-prohibitive costs. The recent CHAMPS trial of the combination of HU and magnesium pidolate in HbSC disease was terminated before adequate data could be collected, but the lack of biological response to magnesium has dampened enthusiasm for this agent (91). However, combinations of hydroxyurea with agents such as senicapoc (Gardos channel blocker) (92), poloxamer 188 (surfactant) (93), rivipansel/GMI-1070 (pan-selectin inhibitor) (94), prasugrel (platelet inhibitor) (95), and regadenoson (adenosine A2A receptor agonist) (96) would seem feasible. The use of decitabine in patients who were refractory to HU has been proposed and needs to be explored, particularly if decitabine is available in an oral formulation (97).

Recommendations for Hydroxyurea Use in SCD

In 2008, a consensus conference at the NIH concluded that HU is efficacious in both children and adults with sickle cell anemia and that it is greatly underutilized (99). New data from the BABY HUG trial showed a similar degree of efficacy of HU to that seen in the MSH trial in adults in reducing the morbidity of the most common vasocclusive complications of sickle cell disease. An important distinction between the BABY HUG and MSH trials is that the former involved subjects who were not selected on the basis of clinical severity. Furthermore, HU has been well tolerated in both adults and children. Although important data still need to be collected, two decades of long-term follow-up has not revealed major safety concerns with the drug. In 2014 the Evidence-Based Management of Sickle Cell Disease Expert Panel Report was published by the NIH/NHLBI (99). The most significant indications for HU treatment in adults with SCA were: ≥ 3 sickle cell-associated moderate to severe pain crises in a 12-month period, pain that interferes with daily
activities and quality of life, severe and/or recurrent acute chest syndrome, and severe symptomatic chronic anemia, all of which were strong recommendations based on moderate to high quality evidence. The recommendations were even broader in children: in infants ≥ 9 months of age, children, and adolescents with SCA, treatment with HU should be offered regardless of clinical severity in order to reduce SCD-related complications (e.g., pain, dactylitis, acute chest syndrome, anemia); this was a strong recommendation with high-quality evidence for ages 9-42 months. The panel strongly encouraged shared decision making and discussion of HU therapy with all patients. In addition, the panel recommended that persons with HbSβ+-thalassemia or HbSC who had recurrent sickle cell-associated pain consult a “sickle cell expert” for consideration of HU therapy. The Guidelines also included a detailed treatment protocol for implementation of HU therapy.

CONCLUSIONS

Since the first trials of hydroxyurea in sickle cell patients with SCD more than 30 years ago, there has been tremendous progress in understanding its mechanisms of action and broadening the scope of its clinical use. However, fundamental questions remain. Although the primary effect of HU is its stimulation of fetal hemoglobin production, the molecular mechanisms of this process are poorly understood and pharmacogenetic influences that may be associated with the wide variability in clinical response are only beginning to be explored. HU’s major benefit occurs from reduction of vaso-occlusive complications, particularly pain events and acute chest syndrome, in both adults and children with SCD, but it is not curative and the hoped for amelioration of organ dysfunction has been realized only to a limited extent. As summarized in Table 3, there is evidence of beneficial effects of HU on CNS, spleen, renal, pulmonary, and retinal function, but, in general, they are not dramatic changes. It remains to be seen if initiation of HU treatment earlier in life or more aggressive dosing will enhance clinical outcomes, increase toxicity, or both. Prospective evaluation of the drug’s effect on mortality is needed. In addition, long-term safety concerns, particularly potential effects on reproduction, need to be explored further. Finally, it is important to recognize that after consideration of all of these factors, the 2014 NIH Expert Panel on Sickle Cell Disease recommended that HU be offered to all very young children with sickle cell anemia (99).
Table 1. Hydroxyurea Interventional Trials – Timeline

<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>HU → ↑ HbF in SCA</td>
<td>Platt (11)</td>
</tr>
<tr>
<td>1992</td>
<td>Phase I-II trial in adults</td>
<td>Charache (25)</td>
</tr>
<tr>
<td>1995</td>
<td>Phase III trial in adults (MSH)</td>
<td>Charache (21)</td>
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<tr>
<td>1998</td>
<td>FDA approval for adults with severe SCA</td>
<td>---</td>
</tr>
<tr>
<td>1999</td>
<td>Phase I-II trial in children (HUGKIDS)</td>
<td>Kinney (26)</td>
</tr>
<tr>
<td>2001</td>
<td>Phase I-II trial in infants (HUSOFT)</td>
<td>Wang (27)</td>
</tr>
<tr>
<td>2011</td>
<td>Phase III trial in infants (BABY HUG)</td>
<td>Wang (23)</td>
</tr>
<tr>
<td>2013</td>
<td>Phase III trial for 2° stroke prophylaxis (and Fe overload) (SWiTCH)</td>
<td>Ware (43)</td>
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## Table 2. Hydroxyurea and Mortality

<table>
<thead>
<tr>
<th>Authors/location</th>
<th>Pt. population/ study design</th>
<th>Genotype/ Inclusion criteria</th>
<th>N</th>
<th>Follow-up period mean/median (range)</th>
<th>Hospital Use</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steinberg, 2010 (MSH)/North America (28)</td>
<td>Adults/ prospective</td>
<td>SCA; MSH cohort; analysis by HU use, not original trial assignment</td>
<td>129/299 of original cohort deceased</td>
<td>Up to 17.5 y</td>
<td>HU for at least 5 y (compared with &lt;5 y) had ↓ mortality</td>
<td></td>
</tr>
<tr>
<td>Voskaridou, 2010 (LaSHS)/Greece (29)</td>
<td>Adults/ prospective</td>
<td>HbSS: 34, HbSβthal: 131, HbSβ+ thal: 165; ≥3 VOC/previous y; CVA or ACS in past 5 y</td>
<td>330 (HU:131 [SCA:87; Sβ+thal:44])</td>
<td>HU: 8 y (0.1-17) No HU: 5 y (0.1-18) ↓; ↓ mortality; ↓ survival: 86% for HU; 65% for non-HU; P = .001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobo, 2013 Brazil (30)</td>
<td>Children/ retrospective</td>
<td>SCD; indications for HU: recurrent VOC; &gt;1 ACS; Hb &lt;6 g/dL; CVA</td>
<td>1760 (HU:267)</td>
<td>7 y (3-17) HU: 2 y (0.1-6.5) ↓50% ↓ mortality; ↓ mortality; 1 death in HU group</td>
<td></td>
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</table>

*Modified from Table 1 of Wong, et al (2).
<table>
<thead>
<tr>
<th>Organ</th>
<th>Type of Study</th>
<th>Patient Population (Age at enrollment or evaluation)</th>
<th>N</th>
<th>Evaluation</th>
<th>Effect of HU</th>
<th>Reference</th>
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<tr>
<td>Spleen</td>
<td>Randomized</td>
<td>9-18 mo (BABY HUG)</td>
<td>193 (½ on HU)</td>
<td>spleen scan</td>
<td>no Δ</td>
<td>Wang 2011 (23)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pit cells</td>
<td>↓</td>
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<td></td>
<td></td>
<td>HJB</td>
<td>↓</td>
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<tr>
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<td></td>
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<td></td>
<td>spleen volume</td>
<td>no Δ</td>
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<td>Observ.</td>
<td>10 yr</td>
<td>43</td>
<td></td>
<td>spleen scan</td>
<td>14% recovery</td>
<td>Hankins 2008 (100)</td>
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<tr>
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<td>3-22 yr</td>
<td>21</td>
<td></td>
<td>spleen scan</td>
<td>↑10, ↓3, stable 8</td>
<td>Santos 2002 (101)</td>
</tr>
<tr>
<td></td>
<td>12.3 yr</td>
<td>12</td>
<td></td>
<td>pit cells</td>
<td>no Δ</td>
<td>Olivieri 1998 (102)</td>
</tr>
<tr>
<td></td>
<td>--</td>
<td>--</td>
<td></td>
<td>HJB</td>
<td>↑</td>
<td>Harrod 2007 (103)</td>
</tr>
<tr>
<td></td>
<td>9.1 yr</td>
<td>40</td>
<td></td>
<td>spleen scan</td>
<td>33% uptake</td>
<td>Nottage 2014 (32)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>Randomized</td>
<td>9-18 mo (BABY HUG)</td>
<td>193 (½ on HU)</td>
<td>DTPA GFR</td>
<td>no Δ</td>
<td>Wang 2011 (23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Alvarez 2012 (33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>urine osmolality</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>urine sp. gr.</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>total kidney volume</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Observ.</td>
<td>adults</td>
<td>26</td>
<td></td>
<td>microalbuminuria</td>
<td>↓</td>
<td>Thompson 2007 (104)</td>
</tr>
<tr>
<td>Observ.</td>
<td>children</td>
<td>9</td>
<td></td>
<td>microalbuminuria</td>
<td>↓</td>
<td>McKie 2007 (35)</td>
</tr>
<tr>
<td>Observ.</td>
<td>35 mo</td>
<td>14</td>
<td></td>
<td>DTPA GFR</td>
<td>stable</td>
<td>Thornburg 2008 (105)</td>
</tr>
<tr>
<td>Observ.</td>
<td>8 yr</td>
<td>3</td>
<td></td>
<td>proteinuria (Enalapril)</td>
<td>↓</td>
<td>Fitzhugh 2005 (34)</td>
</tr>
<tr>
<td>Observ.</td>
<td>7.5 yr</td>
<td>23</td>
<td>GFR microalbuminuria</td>
<td>↓</td>
<td>Aygun 2013 (36)</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>--------</td>
<td>----</td>
<td>---------------------</td>
<td>---</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>(a) 1° Stroke proph.</td>
<td>Randomized</td>
<td>0-18 mo</td>
<td>193 (½ on HU)</td>
<td>TCD velocity</td>
<td>no Δ</td>
<td>Wang 2011 (23)</td>
</tr>
<tr>
<td>(BABY HUG)</td>
<td>Randomized</td>
<td>children on CTX for</td>
<td>TCD velocity</td>
<td>Δ TCD velocity</td>
<td>↓</td>
<td>Ware 2015 (106)</td>
</tr>
<tr>
<td>(TWITCH)</td>
<td>abnormal TCD</td>
<td>TCD velocity</td>
<td>not inferior</td>
<td>↓ velocity</td>
<td>Zimmerman 2007 (40)</td>
<td></td>
</tr>
<tr>
<td>Observ.</td>
<td>abnormal TCD (5.5 yr)</td>
<td>23</td>
<td>clinical course, TCD velocity</td>
<td>no CVA/ 84 pt. yr.</td>
<td>Lefevre 2008 (41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>initiation of HU</td>
<td>24</td>
<td>TCD velocity</td>
<td>↓ vel by 13 cm/sec</td>
<td>Kratovil 2006 (107)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>abnormal TCD</td>
<td>34</td>
<td>clinical course, TCD velocity</td>
<td>↓ vel on HU (200→166)</td>
<td>Gulbis 2005 (47)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cond/abnl TCD</td>
<td>31/19</td>
<td>TCD velocity</td>
<td>↑ vel not on HU (190→200)</td>
<td>Lagunju 2015 (42)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>abnormal TCD</td>
<td>(46)</td>
<td>--</td>
<td>SPIN pilot trial in progress</td>
<td>Galadanci 2015 (44)</td>
<td></td>
</tr>
</tbody>
</table>
### (b) 2° Stroke proph.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Duration</th>
<th>Number</th>
<th>Endpoints</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Randomized</strong></td>
<td><strong>SWiTCH</strong></td>
<td>mean tx hx = 7 yr</td>
<td>66 std. treatment</td>
<td>overt CVA; HU – n=7</td>
<td>Ware 2013 (45)</td>
</tr>
<tr>
<td><strong>Observ.</strong></td>
<td><strong>children with 1° stroke</strong></td>
<td>35</td>
<td>overt CVA</td>
<td>4.6 events/100 pt. yr.</td>
<td>Greenway 2011 (49)</td>
</tr>
<tr>
<td></td>
<td><strong>children with 1° stroke</strong></td>
<td>5</td>
<td>overt CVA</td>
<td>0 events/34 pt. yr.</td>
<td>Sumoza 2002 (44)</td>
</tr>
<tr>
<td></td>
<td><strong>children with 1° stroke</strong></td>
<td>6</td>
<td>overt CVA</td>
<td>2 strokes</td>
<td>deMontalembert 2008 (48)</td>
</tr>
<tr>
<td></td>
<td><strong>children with 1° stroke</strong></td>
<td>8</td>
<td>overt CVA</td>
<td>1 stroke/44 pt. yr.</td>
<td>Gulbis 2005 (47)</td>
</tr>
<tr>
<td></td>
<td><strong>children with 1° stroke</strong></td>
<td>10/33</td>
<td>overt CVA</td>
<td>1/10 on HU (2/100 pt.yr.)</td>
<td>Ali 2011 (50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20/33 not on HU (20/100 pt.yr.)</td>
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</tr>
</tbody>
</table>

### (c) Silent Cerebral Infarcts

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Duration</th>
<th>Number</th>
<th>Endpoints</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Randomized</strong></td>
<td><strong>SIT</strong></td>
<td>10 yr</td>
<td>196</td>
<td>SCI, CVA over 3 yr (CTX vs. Observation)</td>
<td>DeBaun 2014 (51)</td>
</tr>
<tr>
<td><strong>Observ.</strong></td>
<td><strong>Paris cohort</strong></td>
<td>(6.4 yr)</td>
<td>54 nl TCD and MRA → HU</td>
<td>2 abnormal TCD/225 pt. yr. on HU</td>
<td>Bernaudin 2011 (38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13 abnl TCD/nl MRA → CTX→HU</td>
<td>3/13 recurrent abnormal TCD on HU</td>
</tr>
</tbody>
</table>

### (d) Neuro-psych

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Duration</th>
<th>Number</th>
<th>Endpoints</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Randomized</strong></td>
<td></td>
<td>9-18 mo</td>
<td>193 (½ on HU)</td>
<td>Bayley exam</td>
<td>Wang 2011 (23)</td>
</tr>
<tr>
<td><strong>Observ.</strong></td>
<td><strong>SCD children</strong></td>
<td></td>
<td>15 on HU</td>
<td>neuropsych battery</td>
<td>Puffer 2007 (52)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50 not on HU</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cardio-pulmonary</strong></td>
<td><strong>Observ.</strong></td>
<td><strong>male school children</strong></td>
<td>41</td>
<td><strong>HR, time on treadmill</strong></td>
<td>↑ exercise tolerance</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------</td>
<td>--------------------------</td>
<td>----</td>
<td>---------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td><strong>Observ.</strong></td>
<td>children</td>
<td>152 HU</td>
<td></td>
<td>echocardiogram</td>
<td>no Δ TRJ velocity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>247 not on HU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Observ.</strong></td>
<td>children with recurrent ACS</td>
<td>3</td>
<td><strong>O₂ saturation</strong></td>
<td>resolution of hypoxemia</td>
<td><strong>Singh 2008 (108)</strong></td>
</tr>
<tr>
<td><strong>Observ.</strong></td>
<td>SCA 5-21 yr</td>
<td>11</td>
<td></td>
<td><strong>O₂ saturation</strong></td>
<td>O₂ sat Δ = 95→98%</td>
</tr>
<tr>
<td><strong>Review</strong></td>
<td>10 studies</td>
<td><strong>PH/TRJ velocity</strong></td>
<td></td>
<td>inconsistent effect</td>
<td></td>
</tr>
<tr>
<td><strong>Review</strong></td>
<td>2 studies</td>
<td><strong>PH/R heart cath</strong></td>
<td></td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td><strong>Observ.</strong></td>
<td>MSH F/U</td>
<td>17.5 yr F/U</td>
<td></td>
<td>24% pulmonary mortality</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>87% &lt;5 y. HU treatment</td>
<td></td>
</tr>
<tr>
<td><strong>Observ.</strong></td>
<td>LaSHS F/U</td>
<td>5-8 yr F/U</td>
<td></td>
<td>no ↓ in PH mortality</td>
<td></td>
</tr>
<tr>
<td><strong>Growth</strong></td>
<td><strong>Observ.</strong></td>
<td>Hb SS, 5-16 yr</td>
<td>68</td>
<td>serial ht &amp; wt</td>
<td>girls – no Δ with historical cohorts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>boys - ↑ ht &amp; wt</td>
</tr>
<tr>
<td><strong>Randomized</strong></td>
<td>Hb SS, 9-18 mo</td>
<td>193 (½ on HU)</td>
<td>193</td>
<td>serial ht, wt, HC measures</td>
<td>no Δ HU vs. PL</td>
</tr>
<tr>
<td><strong>Priapism</strong></td>
<td><strong>Observ.</strong></td>
<td>adult men</td>
<td>5</td>
<td>clinical course</td>
<td>4/5 benefited</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 yr old male</td>
<td>1</td>
<td>clinical course</td>
<td>correction of ED</td>
</tr>
<tr>
<td><strong>Retinopathy</strong></td>
<td><strong>Observ.</strong></td>
<td>Hb SS, 10-18 yr</td>
<td>123</td>
<td>ophthalm. exam</td>
<td>HbF&lt;15% → 7 fold ↑ in retinopathy; HU helpful</td>
</tr>
</tbody>
</table>
Abbreviations: observ. = observational, HU = hydroxyurea, Δ = change, HJB = Howell-Jolly bodies, DTPA GFR = diethylenetriaminepentaacetic acid glomerular filtration rate, sp. Gr. = specific gravity, TCD = transcranial Doppler ultrasound velocity, CVA = cerebrovascular accident, nl = normal, abnl = abnormal, pt. yr. = patient-years, SCI = silent cerebral infarct(s), CTX = chronic transfusion, comp = comprehension, FSIQ = full scale intelligence quotient, PL = placebo, MRA = magnetic resonance angiography, HR = heart rate, TRJ = tricuspid regurgitant jet (velocity), PH = pulmonary hypertension, F/U = follow-up, HC = head circumference.
References


106. Transcranial Doppler (TCD) With Transfusions Changing to Hydroxyurea (TWiTCH) trial; ClinicalTrials.gov Identifier: NCT01425307.


Despite being described in the medical literature over a hundred years ago, sickle cell disease continues to be associated with significant morbidity and mortality secondary to end-organ damage resulting from ischemia, chronic inflammation, iron overload and endothelial dysfunction. Unfortunately, current therapies for sickle cell disease remain limited, and the only current curative treatment is allogeneic bone marrow transplant, which is associated with significant risks. Impaired nitric oxide bioavailability has been documented in patients with sickle cell disease and has been implicated in the pathophysiology of pulmonary vascular complications and other vasomotor defects, suggesting that therapies aimed at restoring nitric oxide balance may be promising in this patient population. Therapeutic strategies to restore nitric oxide balance in prior clinical trials have included direct inhalation of nitric oxide gas and its donors, amplification of nitric oxide effects through inhibition of cyclic guanosine monophosphate hydrolysis, and oral supplementation with substrates of nitric oxide synthesis. While preclinical trials of nitric oxide-based therapies for the treatment of pulmonary vascular complications in sickle cell disease have been promising, early clinical trials in this patient population have been limited, largely due to the increased risk of painful vasoocclusive crises, which might be attributable to the known dual nociceptive effects of nitric oxide. Further preclinical and clinical trials are warranted to investigate the therapeutic benefits of nitric oxide-based therapies in the treatment of specific complications of sickle cell disease, including pulmonary and systemic hypertension and chronic kidney disease.

Introduction

Known as the first molecular disease, sickle cell anemia was described over a century ago in the English literature by Herrick, who noticed sickle-shaped erythrocytes in the peripheral smear of a patient from Grenada presenting with painful crises. It has been known for over half a century that a single amino acid substitution in the beta subunit is responsible for the polymerization of sickle hemoglobin under conditions of low oxygen tension. However, despite our understanding of the molecular basis of this disease, the only cure currently available for patients is allogeneic bone marrow transplantation, which is associated with significant morbidity and mortality. The pathophysiology of sickle cell disease is complex, and adult patients suffer from a variety of end-organ complications as a result of chronic hemolysis and inflammation, endothelial dysfunction, platelet activation and aggregation, pathologic cellular adhesion, ischemia reperfusion injury and nitric oxide deficiency. Studies have shown that up to fifty percent of patients with sickle cell disease have impaired nitric oxide bioavailability [1], which has been implicated in the pathophysiology of painful vaso-occlusive crises [1,2], pulmonary complications and early mortality [2]. Therefore, there is a clear need for the development of therapeutic agents to restore the physiologic balance of nitric oxide in patients with sickle cell disease. This article will review the development and use of nitric oxide, phosphodiesterase inhibitors and soluble guanylate cyclase (sGC) modulators for the treatment of sickle cell disease in pre-clinical and
clinical trials. Additionally, we will highlight opportunities for the development of new therapies aimed at the restoration of nitric oxide balance.

**NO Function, Synthesis and Metabolism in Sickle Cell Disease**

A highly potent vasodilator, nitric oxide (NO) is expressed by a variety of different cell types (Table 1) and plays an essential role in the regulation of a number of physiologic processes, including smooth muscle relaxation and vasodilation [3], inhibition of platelet aggregation [4], regulation of pro-coagulant protein secretion [5], inhibition of leukocyte-endothelial cell interactions [5], maintenance of vascular integrity and inhibition of endothelial cell damage [6] (Table 2). Intracellular NO synthase (NOS) converts L-arginine to NO and L-citrulline using oxygen and nicotinamide adenine dinucleotide phosphate as substrates [7] (Figure 1). Upon release from endothelial cells, NO activates soluble guanylyl cyclase in the smooth muscle, which results in increased intracellular cGMP, smooth muscle relaxation, vasodilation and increased regional blood flow [3,8]. In sickle cell disease, NO bioavailability is decreased through a number of different mechanisms, including consumption of NO by cell-free hemoglobin [9] and by reactive oxygen species [10], release of arginase into the plasma, which depletes the NOS substrate L-arginine [11,12], and by the release of the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA) [13-18] (Table 3). It has been previously shown that adults with sickle cell disease have impaired arginine and NO metabolism [9,12] and arginine deficiency [12,19], leading to vasculopathy and end organ damage. In addition, prior studies have shown that patients with sickle cell disease have impaired NO-dependent blood flow, which changes minimally with NOS inhibition in half of affected individuals, suggesting baseline vascular endothelial dysfunction in these patients [1,20]. The use of NO for the treatment of sickle cell disease and its complications is therefore of therapeutic interest and has been explored extensively in pre-clinical and clinical trials, although with limited success.

**NO-Based Therapies in Preclinical Trials**

Inhaled nebulized sodium nitrite has been tested in preclinical trials over the past decade or so with promising results. In newborn lambs with pulmonary hypertension, inhaled sodium nitrite demonstrated a sustained reduction in hypoxia-induced pulmonary hypertension and was shown to be immediately converted to NO in measured expiratory gases [21], substantiating its role in the treatment of pulmonary hypertension. Additionally, transgenic mouse models of sickle cell disease showed that inhaled NO attenuates ischemia-reperfusion-induced lung injury [22] and improves survival during hypoxia [23]. The therapeutic benefits of NO and NO donors were further evaluated in a preclinical study of newborn lambs with pulmonary hypertension induced by infusion of free hemoglobin, which showed that inhaled NO and inhaled aerosolized sodium nitrite promote pulmonary vasodilation, unlike intravascular nitrite infusion [24]. These data suggest that inhaled sodium nitrate may be converted to NO within the lung parenchyma by a mechanism that is independent of plasma deoxyhemoglobin [24], and that the use of intravenous NO to induce pulmonary vasodilation may be limited by the NO scavenging activity of cell-free hemoglobin in patients with active hemolysis.

**NO-Based Therapies in Clinical Trials**

Collectively, these preclinical trials substantiated the therapeutic application of inhaled sodium nitrite and inhaled NO for the treatment of human diseases characterized by depleted NO bioavailability, such as sickle cell disease (Table 4). The efficacy of inhaled NO for the treatment of acute chest syndrome had been suggested by several case series [25-27], thereby providing additional support for the investigation of NO-based therapies in human clinical trials of sickle cell disease. Results of a single-institution, prospective, double-blinded, placebo-controlled randomized clinical trial conducted from 1999 to 2001 showed a reduction in hourly pain scores and morphine use at 6 hours with the therapeutic use of inhaled NO for the treatment of vaso-occlusive crises in pediatric patients with sickle cell disease, without any reported toxicity [28]. This set the precedent for an 18 center, double-blinded, randomized placebo controlled trial seven years later in adult patients with vaso-occlusive crises, which showed that inhaled NO significantly reduced pain scores, but not parenteral morphine use, without observed toxicity [29]. However, contrary to the results of prior smaller clinical
trials, a larger phase two, multicenter, randomized, double-blind, placebo-controlled trial of adults with sickle cell disease presenting with vaso-occlusive pain crises showed that inhaled NO did not shorten vaso-occlusive crisis when compared to placebo [30]. Additionally, inhaled NO did not have any beneficial effects on the secondary end points of the study, including length of hospitalization, change in pain scores or total opioid use [30].

Topical sodium nitrite has shown early promise in the treatment of chronic leg ulcers in adults with SCD [31]. In a phase 1, open label dose escalation study, a dose-dependent effect was seen in ulcer healing, and in resolution of ulcer pain. Application of topical sodium nitrite induced significant increase in regional cutaneous blood flow detected by both infrared thermography and laser speckle contrast imaging.

**Limitations of NO for the Treatment of Sickle Cell Disease and Potential Future Applications**

Although the safety of inhaled NO has been demonstrated in multiple clinical trials and it has already been approved by the Food and Drug Administration for the treatment of pulmonary hypertension in newborn infants [9], there are no formal indications at this time for its therapeutic use in the treatment of sickle cell disease and its complications. The failure of a large, multicenter, double-blinded, randomized control trial to show a clinical benefit of inhaled NO was both surprising and disappointing, as preclinical trials [21], and intravascular nitrite is already accepted for use as an antidote for cyanide poisoning [34], with demonstrated safety and tolerability in humans. Given that NO has a half-life lasting only seconds, another possibility for its limited efficacy in human clinical trials is that its short half-life prevented effective drug delivery from the lungs to ischemic tissues during vaso-occlusive pain crises. Alternatively, it is also possible that ischemic injury to tissue is not reversible in vaso-occlusive pain crises by the time it is recognized clinically.

In multiple human randomized clinical trials, end points such as pain scores, use of parenteral morphine, length of hospitalization and time to crisis resolution were used to study the efficacy of NO-based therapies in the treatment of vaso-occlusive pain crises. However, use of these endpoints may be problematic, as multiple studies have shown conflicting results regarding the role of NO and cyclic guanosine monophosphate (cGMP) in pain signaling [35]. Nitric oxide is produced in the spinal dorsal horn neurons in response to painful stimuli [36], and multiple preclinical studies have supported a pro-nociceptive role of NO in the dorsal root ganglia. For example, early animal studies suggested that intrathecally administered NOS inhibitors may reduce inflammatory and neuropathic pain [37,38]. Additionally, more recent animal studies have shown that inhibition of tetrahydrobiopterin (BH4) synthesis, an essential cofactor for NO production, reduces inflammatory and neuropathic pain [39], and SCD patients with genetic variants in a BH4 synthetic gene experience less pain [40]. Furthermore, intrathecally administered NO donors and cGMP analogues have been shown to increase hyperalgesia [41,42]. While it has been reported that high doses of NO cause hyperexcitability and cGMP-dependent hyperalgesia [43], low doses have been reported to reduce hyperalgesia [44]. Preclinical data have also demonstrated that NO inhibits no-ception in the peripheral and central nervous systems and that NO potentiates the analgesic effect of opioids [45], thereby supporting a dual role for NO on pain signal transduction. Given the emerging evidence for dose-dependent effects of NO on nociception, dose titration studies in humans may provide useful information for future therapeutic applications. In addition, endpoints other than pain should be considered for future preclinical and clinical studies of NO in the treatment of sickle cell disease, such as prevention and treatment of pulmonary

**Note:** The text continues from here.
hypertension and other vascular complications. Given that NO is known to inhibit platelet aggregation and secretion of procoagulant proteins, there may be a role for NO in the prevention of thrombosis as well, which is known to occur at a higher incidence in patients with sickle cell disease compared to healthy controls. Finally, optimal routes of NO administration and the use of NO donors for the treatment of sickle cell disease need to be explored further in preclinical and clinical trials.

**Sildenafil Preclinical Results**

Another strategy that has been employed to amplify the effects of endogenous NO is the use of sildenafil, a selective vasodilator which inhibits hydrolysis of cGMP by phosphodiesterase 5, an enzyme expressed within the vasculature of the penis, lungs, and sinus mucosa [46]. By inhibiting breakdown of cGMP, a downstream signal transducer of NO, sildenafil prolongs its intracellular effects and incites a series of signaling events leading to smooth muscle relaxation (Figure 1) [9]. While its use for the treatment of penile erectile dysfunction has been well-established, preclinical and clinical trials have suggested a role for sildenafil in the treatment of pulmonary hypertension, a known cause of morbidity in sickle cell disease [47] which has been associated with a two-year mortality rate as high as 50% [48]. The potential therapeutic benefit of sildenafil for the treatment of acute pulmonary hypertension was noted over a decade ago in preclinical trials of lambs, in which sildenafil induced dose-dependent reductions in pulmonary arterial pressure and pulmonary vascular resistance without prolonging the vasodilatory effects of inhaled NO [49]. A subsequent preclinical trial in sheep demonstrated the ability of nebulized sildenafil to augment the pulmonary vasodilatory effects of inhaled NO [49]. The benefits of sildenafil in the treatment of primary pulmonary hypertension in humans were suggested in pediatric and adult case reports which documented improvements in pulmonary hemodynamics and exercise capacity [50,51].

**Sildenafil in Clinical Trials**

These preclinical and clinical observations set the precedent for a small clinical trial which demonstrated reductions in mean pulmonary arterial pressure and pulmonary vascular resistance with sildenafil treatment in adults with primary pulmonary hypertension, an effect which was further augmented after iloprost inhalation [52]. Subsequent case series and case reports demonstrated beneficial effects of sildenafil in the treatment of pulmonary hypertension secondary to thalassemia [53] and lung fibrosis [54], post-operative pulmonary hypertensive crises [55] and primary pulmonary hypertension [56-58]. Less than a decade ago, the therapeutic benefits of sildenafil for the treatment of severe pulmonary hypertension secondary to hemoglobinopathies was demonstrated in a small, open-label multicenter trial of seven adult patients with thalassemia intermedia, thalassemia major and sickle thalassemia, in which sildenafil significantly decreased pulmonary pressures and improved tricuspid gradients and exercise capacity without any significant adverse events [59]. Another open-label study of twelve adult patients with sickle cell disease and secondary pulmonary hypertension also showed reductions in pulmonary arterial systolic pressures and a twenty percent increase in the distance walked at six minutes, indicating improvements in exercise capacity and cardiopulmonary reserve [47]. While transient headaches and eye-lid edema were noted in several patients, none of the male patients were noted to develop priapism [47], although the patients in this study were notably at low risk for priapism, and one patient had a prior diagnosis of erectile dysfunction. Subsequently, a non-randomized, multicenter study demonstrated improvements in pulmonary pressures and six minute walk distances with sildenafil but not L-arginine in twenty-seven adults with stable sickle cell disease on hydroxyurea, thereby substantiating the results of prior clinical trials [60]. In addition, sildenafil, but not L-arginine, was noted to increase hemoglobin F (HbF) levels in subjects with stable baseline HbF levels [60].

Based on the promising results of these uncontrolled case series, a randomized multicenter, double-blinded, placebo-controlled trial of seventy-four adolescent and adult patients with sickle cell disease with elevated tricuspid regurgitation velocity and decreased exercise capacity was designed to study the efficacy of sildenafil [61]. The study was terminated early, because the subjects treated with sildenafil experienced a higher rate of pain requiring hospitalization. Furthermore, there was no evidence of a benefit of sildenafil on six minute walk distance or Doppler-estimated right ventricular systolic pressures compared to placebo. The early closure due to safety concerns resulted in inadequate statistical power to detect the primary endpoint.
Anecdotal case reports supported a potential role of sildenafil in the treatment of sickle cell priapism [62,63]. These reports led to a randomized, single center, double-blind, placebo-controlled clinical trial to investigate the efficacy of sildenafil for the treatment of sickle cell disease-related priapism [64]. This study unfortunately only accrued 25% of the desired number of patients, and did not show a significant difference in the primary endpoint, fifty percent reduction in priapism episodes, between the sildenafil and placebo groups by intention-to-treat and per-protocol analyses. However, at the end of the open-label phase, approximately one third of patients had reported a reduction in priapism frequency with sildenafil treatment by either analysis [64], suggesting a potential benefit consistent with the results of preclinical studies and clinical anecdotal case series.

Limitations of Sildenafil for the Treatment of Sickle Cell Disease and Potential Future Applications

Similar to NO, sildenafil unfortunately did not show a clinical benefit for the treatment of pulmonary hypertension secondary to sickle cell disease in a large, multicenter, randomized, double-blinded, placebo-controlled trial. These results were disappointing, given the promising preclinical data and case series which had suggested a potential benefit of sildenafil for the treatment of pulmonary hypertension. As mentioned above, one potential reason for the failure of this study to demonstrate a clear benefit of sildenafil is that the study was inadequately powered to detect the primary endpoint (change in six minute walk test distance) as estimated by futility analysis due to its early termination. It is possible that the increased painful episodes in the sildenafil arm were not observed in prior open-label studies due to the lack of controls. In one of these open-label studies, all of the subjects had stable disease on maximal hydroxyurea therapy [60], which could potentially explain the absence of painful episodes observed. While it is plausible that the increased pain experienced by patients receiving sildenafil may have limited their ability to perform the six minute walk test, a post-hoc analysis did not reveal a correlation between pain scores and six minute walk distance. However, at six weeks, more subjects in the sildenafil treatment arm experienced dyspnea with overall higher tricuspid regurgitation velocities (TRV) and pro-BNP levels, suggesting that there may have been additional factors interfering with the ability of these subjects to complete the six minute walk distance test. Additionally, not all of the patients in the study had confirmation of pulmonary hypertension by right heart catheterization, which may limit the applicability of these results to patients with hemodynamically confirmed pulmonary hypertension. In fact, among the subjects with a tricuspid regurgitation velocity greater than 3.0 m/s who underwent right heart catheterization, almost half of them did not have pulmonary hypertension as defined by a mean pulmonary arterial pressure greater than 25 mmHg [61]. The approach to patients with pulmonary artery pressure that is above normal but below the diagnostic criterion for pulmonary hypertension remains controversial.

The increase in painful episodes requiring hospitalization in the sildenafil treatment arm was an unexpected finding. Prior studies have suggested that NO donors and cGMP analogues may increase hyperalgesia [37,41,42,65], thereby suggesting a potential mechanism by which sildenafil, an inhibitor of cGMP hydrolysis, may increase pain transduction within the nervous system. Interestingly, a preclinical study demonstrated that intrathecal administration of sildenafil decreased pain threshold in rats with nerve injury induced by nerve ligation, but not in control rats that did not undergo nerve ligation [66]. A potential explanation for this discrepancy is that sildenafil may increase the total amount of cGMP at the spinal cord and therefore amplified nociceptive responses exclusively in the rats that underwent prior nerve ligation. This putative mechanism of sildenafil-mediated nociception within the central nervous system is further supported by preclinical models of inflammatory and neuropathic pain which suggest that the NO-cGMP pathway plays a role in the nociceptive responses of spinal dorsal horn neurons in hyperalgesic states [65,67].

Despite the disappointing results of sildenafil for the treatment of sickle cell disease-related pulmonary hypertension in a large, randomized, placebo-controlled trial, there may still be a role for sildenafil in the treatment of patients who have been stabilized with hydroxyurea or transfusion therapy. However, further randomized studies are necessary to determine if this approach could successfully reduce painful episodes induced by sildenafil. Although hydroxyurea therapy alone has reduced tricuspid regurgitation velocity in anecdotal cases of SCD patients [68,69], it is not been associated with reductions in
tricuspid regurgitation velocities in large cross-sectional studies [2,70], suggesting the need for the identification of additional therapies for the treatment of sickle cell disease-associated pulmonary hypertension, perhaps using a multi-therapy approach. Additionally, further investigation is necessary in preclinical and clinical studies to clarify the role of sildenafil in pain transduction, as pain was the major adverse effect limiting the use of sildenafil for the treatment of secondary pulmonary hypertension in a large human clinical trial. Preclinical data have shown that NO may have antagonizing effects on nociception in a dose-dependent manner [43,44], and possibly dose titration studies for sildenafil might further clarify its role in pain transduction as well.

One of the perceived limitations of using sildenafil for the treatment of sickle cell disease and its complications has been the concern that it may induce priapism, which is a known vascular complication of the disease. However, recent evidence has suggested that priapism frequency may be reduced in patients who are adherent to sildenafil therapy [64], suggesting that it may be safe for the treatment of male patients with sickle cell disease. Finally, sildenafil may have therapeutic applications for the treatment of other complications of sickle cell disease in addition to pulmonary hypertension. For example, a recent study of twelve subjects with sickle cell disease complicated by elevated TRV demonstrated a reduction in platelet activation in patients taking oral sildenafil [71], thereby suggesting a putative role for sildenafil in the prevention and treatment of thrombosis in sickle cell anemia, which is recognized as a disease of chronic hemostatic activation [71], especially in SCD patients with high TRV [72]. Trials of anti-platelet therapies for SCD may be more appropriate than investigation of sildenafil for this purpose.

Arginine, Substrate for Nitric Oxide Synthesis

L-arginine, the obligate substrate for nitric oxide synthase, is deficient in plasma of patients with SCD, apparently due to consumption by ectopic localization of erythrocyte arginase-1 as another consequence of intravascular hemolysis [7,12]. Arginine supplementation in sickle cell mice improves Gardos channel dysfunction, red cell density, hemolysis, oxidative stress and motor coordination [73-75]. Arginine depletion is further exacerbated at times of vaso-occlusive pain crisis [19]. An early phase clinical trial showed preliminary evidence that arginine supplementation during vaso-occlusive crisis can shorten its duration and reduce opioid requirements [76], and a follow up trial is under way.

Soluble Guanylate Cyclase Modulators for the Treatment of Sickle Cell Disease Vasculopathy

Nitric oxide is a regulator of vascular function through activation of the enzyme soluble guanylate cyclase (sGC) within the vessel wall of smooth muscle cells, which then converts guanosine triphosphate to cGMP. As explained earlier, in sickle cell disease the canonical NO signaling is disturbed at multiple steps and many adults with sickle cell disease go on to develop pulmonary hypertension (PH) [2].

One might hypothesize that therapies to target the downstream mediators within this pathway may recover vascular function. The conceivable treatment of conditions in which NO levels are deficient would have great therapeutic promise. Recently, a new class of treatments has been developed that bypass issues of NO bioavailability termed "sGC Modulators". These small molecules are able to directly bind to sGC, and increase intracellular levels of the messenger cGMP, which leads to decreased vascular tone [77]. One compound is heme-dependent and it stimulates the cyclase enzyme’s sensitivity to even very small quantities of NO that remain available, and adds a synergistic effect when NO binds [78]. Another compound is heme-independent and has the ability to activate an oxidized form of soluble guanylate cyclase, which contains either a ferric heme or has lost the heme moiety entirely. This compound activates the enzyme independently of NO additive to endogenous NO binding.

Preclinical in vivo models of kidney and cardiovascular disease and PH have shown promising effects of both sGC stimulators and sGC activators on fibrotic changes, cardiac remodeling, vascular function, NO sensitivity and platelet activation [79,80]. Three recent studies have demonstrated the success of the stimulator compound riociguat. Riociguat improved 6-minute walk distance in patients with idio-pathic pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension (Type 4 PH) [81,82]. In those studies the serious adverse events involving syncope, hypotension and acute renal failure occurred in less than 2% of patients. Subsequently, riociguat improved cardiac index and stroke volume with reduction in mean pulmonary arterial blood pressure in patients with PH secondary to systolic left ventricular dysfunction (Type 2 PH); which was supported by a phase
Ilb double-blind, randomized, placebo-controlled trial [83]. A trial of oral riociguat in adults with SCD has been registered, but is not yet enrolling patient at the time this manuscript was submitted [84].

Sickle cell anemia is a disease where a significant amount of intravascular hemolysis exists, which depletes levels of NO through consumption by the hemoglobin-dioxygenation reaction [1,85]. The oxidative stress increases within the vascular space yielding oxidized heme moieties in a portion of the smooth muscle cell sGC, which becomes unresponsive to exogenous/endogenous NO and sGC stimulators. Rats infused with cell-free hemoglobin developed vasoconstriction with impaired NO signaling that was not rescued by either the NO donor sodium nitroprusside or sildenafil. However, the vasoconstriction was attenuated with sGC modulators functioning independently of NO, which restored cGMP mediated vasodilation [86]. The capability of the novel sGC activator molecule to bypass NO signaling and bind directly to the oxidized sGC enzyme increasing intracellular cGMP, providing vasorelaxation offers a potential therapeutic benefit and may be advantageous for impaired vascular tone in conditions that are associated with oxidation of sGC, such as hemolytic diseases.

Other Agents Affecting NO Signaling

The results of these clinical trials and a few other NO-related agents tested in early phase clinical trials are summarized in Table 4, including simvastatin, which can stimulate NOS [87], tetrahydrobiopterin, a cofactor for NOS [88]. Additional NO-related agents with published preclinical results are summarized in Table 5, including an inhibitor of phosphodiesterase 9A. PDE9A hydrolyzes cGMP, and its inhibition can amplify NO signaling in tissues that express PDE9A [89], especially neutrophils from patients with SCD [90].

Conclusions

While the molecular basis of sickle cell disease has been known for over one hundred years, patients continue to suffer from end-organ complications and increased morbidity as a result of chronic hemolysis and inflammation, endothelial dysfunction and nitric oxide deficiency, the latter of which has been implicated in the pathophysiology of pulmonary complications and vasculocclusive crises. Nitric oxide deficiency has been noted in up to fifty percent of patients with sickle cell disease, and the safety and efficacy of nitric oxide-based therapies for the treatment of primary pulmonary hypertension and penile erectile dysfunction has been previously demonstrated in human clinical trials, thereby providing the rationale for the investigational use of these therapies for the treatment of vascular complications in sickle cell disease. While nitric oxide-based therapies have been shown to reduce pulmonary pressures effectively and safely in animal models, application of these therapies for the treatment of sickle cell disease has been limited in large multi-center human clinical trials as a result of increased painful episodes. Preclinical data have suggested a dual, conflicting role of nitric oxide and cyclic guanosine mono-phosphate in pain signal transduction, underscoring the need for additional studies to clarify the role of nitric oxide signaling pathways in nociception in order to optimize future therapies. Additionally, the use of soluble guanylate cyclase modulators to stimulate downstream cyclic guanosine monophosphate production independently of nitric oxide availability may have therapeutic benefit for the treatment of vascular complications in sickle cell disease, as well as other disease states characterized by oxidative stress, impaired nitric oxide bioavailability and vasculopathy.

Acknowledgments

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References


Table 1. Cells Known to Produce Nitric Oxide [4]

<table>
<thead>
<tr>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular endothelial cells</td>
</tr>
<tr>
<td>Neurons</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
</tr>
<tr>
<td>Macrophages</td>
</tr>
<tr>
<td>Neutrophils</td>
</tr>
<tr>
<td>Platelets</td>
</tr>
<tr>
<td>Pulmonary epithelial cells</td>
</tr>
</tbody>
</table>

Table 2. Downstream Physiological Effects of Nitric Oxide

<table>
<thead>
<tr>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth muscle relaxation, vasodilation and increased regional blood flow [3]</td>
</tr>
<tr>
<td>Inhibition of platelet aggregation [4]</td>
</tr>
<tr>
<td>Regulation of the expression of endothelial cell adhesion molecules [91]</td>
</tr>
<tr>
<td>Inhibition of pro-coagulant protein secretion [5]</td>
</tr>
<tr>
<td>Regulation of vascular smooth muscle cell proliferation [5]</td>
</tr>
<tr>
<td>Inhibition of endothelial-leukocyte interactions and vascular inflammation [5]</td>
</tr>
<tr>
<td>Maintenance of vascular integrity and inhibition of endothelial cell damage [6]</td>
</tr>
<tr>
<td>Limitation of ischemia-reperfusion injury [92]</td>
</tr>
<tr>
<td>Increases the oxygen affinity of sickled erythrocytes [93]</td>
</tr>
</tbody>
</table>

Table 3. Mechanisms of Impaired Nitric Oxide Bioavailability in Sickle Cell Disease

<table>
<thead>
<tr>
<th>Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consumption of NO by cell-free plasma hemoglobin [9]</td>
</tr>
<tr>
<td>Consumption of NO by reactive oxygen species [10]</td>
</tr>
<tr>
<td>Depletion of plasma arginine by arginase released from lysed red cells [11,12]</td>
</tr>
<tr>
<td>Endogenous NOS inhibitor asymmetric dimethylarginine (ADMA) released from lysed red cells [13-18]</td>
</tr>
</tbody>
</table>
Table 4. Summary of clinical data for investigational agents for SCD involving nitric oxide or phosphodiesterase inhibition.

<table>
<thead>
<tr>
<th>Medication</th>
<th>Mechanism(s) of Action</th>
<th>Indication(s)</th>
<th>Outcome of Prior Preclinical + Clinical</th>
<th>Potential Side Effects or Limitations</th>
<th>FDA Approved?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2. Improvement of ventilation-to-</td>
<td></td>
<td></td>
<td>2. Administration requires special handling</td>
<td></td>
</tr>
<tr>
<td>Intravenous sodium</td>
<td>Converted to NO by deoxyhemoglobin at acidic pH and low oxygen tension [94]</td>
<td>Formal indications not yet established</td>
<td>Promoted vasodilation and improved regional blood flow in a phase II/III clinical trial [94]</td>
<td>Transient nausea reported by one patient at the highest dose of nitrite [94]</td>
<td>Accepted</td>
</tr>
<tr>
<td>nitrite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>as an intravenous antidote for cyanide poisoning in humans [9], but not as an inhaled nebulized therapy for sickle cell</td>
</tr>
<tr>
<td>Topical sodium nitrite</td>
<td>1. NO donor [31]</td>
<td>Formal indications not yet established</td>
<td>Increased per-wound blood flow and decreased leg ulcer size and pain</td>
<td>Two patients noted to have asymptomatic decreases in diastolic blood pressure [31]</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>2. Enhances blood flow in ulcers [31]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Has known bac</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Oral tetrahydrobi-</td>
<td>1. Essential cofactor for endothelial NO synthase [95,96]</td>
<td>Formal indications not yet established</td>
<td>- Preliminary evidence of improvement in endothelial dysfunction in SCD patients with abnormal endothelial</td>
<td>No adverse effects noted in a Phase 2 study in SCD</td>
<td>Approved</td>
</tr>
<tr>
<td>opterin (R-BH4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>for the treatment of patients with phenylketonuria, but not currently for sickle cell disease</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>- Activation of HgbF expression [97]</td>
<td>Indicated for adults to decrease severe painful episodes, hospitalizations, number of blood transfusions, and acute chest syndrome [102]</td>
<td>- Reduction in the severity and duration of vasoocclusive pain crises [28]</td>
<td>- Routine monitoring of CBC required due to myelosuppressive effects</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>- Reduction of HgbS polymerization [98]: May play a role as an NO donor based on in vitro and animal studies [99-101]</td>
<td></td>
<td>- 40% reduction in mortality rate compared to placebo [98]</td>
<td>- Neutropenia may limit dose titration</td>
<td></td>
</tr>
<tr>
<td>Oral L-Arginine</td>
<td>1. Improvement in NO bioavailability [9]</td>
<td>Formal indications not yet established</td>
<td>- May increase NO when given in conjunction with hydroxyurea [11]</td>
<td>- High doses are necessary in order to overcome the impact of arginine and asymmetric dimethylarginine on arginine bioavailability</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- In pilot study, increased plasma NO metabolites and</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Co-administration of</td>
<td></td>
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</table>

- In children with SCD and vasoocclusive crises [76]
| **Statins** | 1. HMG coA reductase inhibition  
2. Increase sensitivity to NO  
3. Inhibit expression of cell adhesion molecules and protect against increased tissue factor expression [104] | Treatment of hyperlipidemia  
- Limited improvement in NO-dependent blood flow in SCD [105]  
- In pilot study in SCD children, simvastatin increased NO metabolites and decreased IL-6 and C-reactive protein [87] | Long term effects in patients with SCD unknown | Approved for treatment of hyperlipidemia, but not for sickle cell disease. |
| **Sildenafil** | 1. Inhibits hydrolysis of cGMP by PDE5, prolonging cGMP effect which results in smooth muscle relaxation [9]  
2. Pulmonary hypertension | - May promote pain sensitivity in SCD, but no clear benefits on vasculopathy or CNS injury [106]  
- Decreased pulmonary artery systolic pressure and increased 6 min walk distance in 12 patients with SCD and PH [47]  
- No priapism noted over 6 months in 3 male patients [47] | - In 12 patients with SCD and PH, some patients developed eye lid edema and headache [47] | Indicated for the treatment of priapism and pulmonary hypertension, but not yet formally indicated for SCD |
| **Riociguat** (BAY 63-2521) | soluble guanylate cyclase stimulator [81]  
- Pulmonary arterial hypertension [81]  
- Chronic thromboembolic pulmonary hypertension [82] | Not yet tested in SCD | Not yet tested in SCD | Yes for pulmonary hypertension, but not formally indicated for SCD |
| **Allopurinol** | 1. Inhibits xanthine oxidase from producing uric acid and reactive oxygen species | Treatment of gout  
None available for patients with SCD | Long term effects in patients with SCD unknown | Yes, for the treatment of gout, but not formally indicated for SCD |
| **Oral citrulline** | 1. Precursor for the synthesis of endogenous L-arginine [6]  
Formal indications not yet established | - Symptomatic improvement, increased plasma arginine levels, and reductions in leukocyte count observed in phase II trials [6]  
- Reduction in plasma ferritheme levels | Long term effects in patients with SCD unknown | No |
Table 5. Summary of investigational agents involving nitric oxide or phosphodiesterase inhibition in preclinical research for SCD.

<table>
<thead>
<tr>
<th>Medication</th>
<th>Mechanism(s) of Action</th>
<th>Potential Indication(s) for Patients with Sickle Cell</th>
<th>Outcome of Prior Preclinical Trials</th>
<th>Toxocities Observed in Preclinical Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAY 73-6691</td>
<td>- Phosphodiesterase 9 inhibitor (PDE9) [90]</td>
<td>- Could potentially have a role in the prevention of vasooclusive crises in patients with sickle cell disease in conjunction with hydroxyurea</td>
<td>- PDE9A expression was significantly higher in the reticulocytes and neutrophils of SCD patients compared to healthy controls [90]</td>
<td>- Long-term effects in patients with SCD unknown (currently undergoing clinical trials for the treatment of Alzheimer’s Disease) [89,109]</td>
</tr>
<tr>
<td></td>
<td>- Regulation of intracellular cGMP and cAMP levels [90]</td>
<td></td>
<td>- BAY 73-6691 significantly increased production of the γ-globin gene in K562 cells and reversed the increased adhesive properties of SCD neutrophils [90]</td>
<td>- Relatively high tissue-specific expression of PDE9 in hematopoietic cells may limit systemic toxicity in humans [90,108]</td>
</tr>
<tr>
<td>Inhaled nebulized sodium nitrite</td>
<td>1. NO donor [9]</td>
<td>- Could potentially have a role in the treatment of symptomatic pulmonary hypertension in patients with sickle cell disease</td>
<td>More effective and longer lasting reduction in pulmonary pressures compared to inhaled NO [21]</td>
<td>Toxicity studies in humans are ongoing [9]</td>
</tr>
<tr>
<td></td>
<td>2. Oxidization of cell-free plasma hemoglobin, leading to a reduction of NO scavenging and pulmonary vasodilatation</td>
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</tbody>
</table>

**Figure 1: Physiologic Roles of Nitric Oxide.** Nitric oxide (NO) is a potent vasodilator produced by vascular endothelial cells from the amino acid precursor, L-arginine. Nitric oxide plays an essential role in the regulation of numerous physiologic processes that collectively improve regional blood flow, including: smooth muscle relaxation and vasodilation [3], inhibition of vascular smooth muscle cell proliferation [5], inhibition of platelet aggregation [4] and pro-coagulant protein secretion [5], inhibition of leukocyte-endothelial cell interactions [5], regulation of adhesion molecule expression in endothelial cells [91], limitation of ischemia-reperfusion injury [92], inhibition of vascular endothelial cell damage [6] and improvement in the oxygen affinity of sickled erythrocytes [93]. Disruption of the physiologic functions of nitric oxide contributes to the development of vasculopathy in sickle cell disease, which is characterized by chronic hemolysis and impaired nitric oxide bioavailability.

**Figure 2: Disruption of Nitric Oxide Homeostasis in Sickle Cell Disease.** In sickle cell disease, nitric oxide deficiency occurs by several different mechanisms: (A) Cell-free hemoglobin is released into the plasma as a result of chronic intravascular hemolysis and consumes nitric oxide, thereby increasing oxidative stress. (B) Cell free hemoglobin promotes the oxidation of tetrahydrobiopterin (BH4), an essential cofactor for endothelial nitric oxide synthase, to dihydrobiopterin (BH2), leading to impaired enzymatic function and decreased nitric oxide production. (C) In addition to cell free hemoglobin, additional factors
contribute to increased oxidative stress burden in sickle cell disease, including ischemia reperfusion injury as a result of vaso-occlusive episodes and increased superoxide production by xanthine oxidase and NADPH oxidase. Like cell free hemoglobin, reactive oxygen species also scavenge nitric oxide and exacerbate nitric oxide deficiency in sickle cell disease. (D) Hemolysis of sickled erythrocytes leads to the release of arginase extracellularly which depletes L-arginine, the amino acid precursor for nitric oxide synthesis. (E) Multiple factors increase nitric oxide bioavailability by promoting the reduction of nitrite to nitric oxide, including: acidosis, xanthine oxidase, deoxyhemoglobin, deoxymyoglobin, respiratory chain enzymes and ascorbic acid.

**Figure 3: Putative Therapeutic Targets for the Treatment of Sickle Cell Disease Associated Vasculopathy.** Multiple investigational nitric oxide-based therapies have been used in preclinical and human clinical trials for the treatment of vasculopathy in sickle cell disease, with varying results. (A) Inhaled NO has been investigated for the treatment of vaso-occlusive crises in sickle cell disease, without a beneficial effect on painful crises in a large, multicenter human clinical trial [30]. (B) Intravenous sodium nitrite and topical sodium nitrite are NO donors which have been shown to increase regional blood flow in early phase clinical trials. (C) Hydroxyurea may play a role as an NO donor and decrease the expression of cellular adhesion molecules based on preclinical data. (D) Statin therapy may increase sensitivity to NO and also decrease the expression of cellular adhesion molecules. (E) Hemoglobin promotes the reduction of nitrite to NO. (F) BAY 73-6691 is a phosphodiesterase 9 inhibitor which improves NO responsiveness by preventing the breakdown of cGMP. BAY 73-6691 also decreases interactions between sickled erythrocytes and leukocytes. (G) Sildenafil is a phosphodiesterase 5 inhibitor, which also inhibits hydrolysis of cGMP and prolongs NO effect. (H) Oral L-arginine is the amino acid precursor for nitric oxide synthesis. i. Allopurinol inhibits the production of reactive oxygen species by xanthine oxidase, which may reduce consumption of nitric oxide. (J) L-citrulline is a precursor for the synthesis of L-arginine and may improve nitric oxide bioavailability.
Figure 1
Figure 2

Nitric Oxide Homeostasis

[Diagram showing the processes involved in nitric oxide homeostasis, including pathways for nitric oxide production and regulation.]
Figure 3
SECTION II
Insights into the Worldwide Clinical and Social Consequences of Sickle Cell Disease
Social and Spiritual/Religious Issues Confronting African Americans with Sickle Cell Disease

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Abstract

BACKGROUND: People with SCD are embedded in overlapping social networks (that include family, friends, medical professionals, God) that can affect coping. Eight men and 16 women with SCD were interviewed to provide preliminary information about the types of social and spiritual/religious issues that they confront in living with SCD. All the participants were African Americans.

RESULTS: Participants identified what was helpful or unhelpful in how others interacted with them as well as the stereotypes that others held about SCD. Participants also described the different forms of prayer and communication with God that they used to cope with SCD.

CONCLUSIONS: The results illustrate how cultural, social/interpersonal and spiritual/religious contexts affect living with SCD. Being the recipient of helpful or unhelpful social interactions, being the target of stereotyping, and engaging in spiritual/religious activities are important issues in coping with SCD. Future research might examine how these contextual factors affect quality of life and health outcomes among SCD patients.

Sickle cell disease (SCD) refers to a group of inherited blood disorders associated with defects in the red blood cell protein (hemoglobin) that transports oxygen to tissues in the human body. Blood cells that contain sickle cell hemoglobin are deformed and take the shape of a C-shaped farm tool (a sickle) after they lose oxygen. These sickle cells stick together, making it difficult for them to move through blood vessels. The primary clinical symptom associated with SCD involves episodes of sickle cell pain that are caused by red blood cell vaso-occlusion, meaning the blockage of blood to a particular area of the body.¹

SCD pain is a frequent and, for many patients, even a daily experience.² Blockage of small arteries and capillaries by the sickle-shaped blood cells may cause a variety of health problems, including severe infections and damage to internal organs, bones, and tissues. These health problems associated with sickle cell disease (including episodes of severe pain) will require the use of the health care system, such as visits to primary care providers, emergency rooms, and hospitalizations. On the other hand, many individuals with SCD often underutilize health care in response to sickle cell-related problems, preferring to cope with severe sickle cell pain on their own.³

A variety of medical interventions are available for treating sickle cell disease, including the use of over-the-counter and prescription pain medications, blood transfusions, and drugs such as hydroxyurea that help prevent the formation of sickle-shaped red blood cells. In addition to medical interventions for SCD, most psychological interventions (including relaxation training, cognitive coping strategies, social support groups, and patient education) focus on promoting skills to reduce the risk of pain episodes and to cope with pain episodes when they occur.⁴ The only recognized cure at the present time for sickle cell disease involves bone marrow or stem cell transplants, but these procedures are risky and may have serious side effects, including death.¹,⁵ Sickle cell disease is no longer viewed as a childhood illness where few people with this disease were expected to live past childhood. Nevertheless, despite medical advances, many persons with the most common and severe form of sickle cell disease, sickle cell anemia, might be expected to live only into their late 40s or 50s in the United States.⁶,⁷

Different groups of individuals may be afflicted with sickle cell disease, including those with ancestors from the Mediterranean, Middle East, and Asia. However, among the estimated 89,000 people with SCD in the United States, most persons with SCD are of African descent.⁶ SCD is found in approximately 1 of 500 births among African Americans and sickle cell trait occurs among 1 in 12 African Americans. Sickle cell trait means that someone has inherited a sickle cell gene from one parent and a “normal” gene from the other parent.⁹ The severest form of
sickle cell disease involves the inheritance of sickle cell genes ("S") from each parent. This form of sickle cell disease is called sickle cell anemia (Hb SS). Other types of sickle cell disease include two types of sickle cell-beta thalassemia ("o" and "+"), and sickle cell hemoglobin C (SC). Individuals with HbS beta-thalassemia have a severe type of sickle cell disease, whereas those with sickle cell hemoglobin C and HbS beta + thalassemia tend to have a milder type of the disease.1,5,10

Given that the median life expectancy for someone with sickle cell disease now extends well into adulthood,1,6,7 patients with this disease are likely to confront a variety of medical, developmental, and social challenges. These issues include managing and treating various health problems associated with a chronic disease, such as the recurrence of unpredictable bouts of sickle cell-related pain and numerous medical complications that might be associated with sickle cell disease. Coping with these medical problems involves repeated interactions with physicians, nurses, and other health professionals over the life span. Coping with sickle cell disease-related health problems also adds to the complexities of dealing with other life challenges, such as friendships, intimate partnerships, parenting, education, work, and religious/spiritual beliefs.

Based on interviews with individuals affected with SCD, we aim to shed light on the social/interpersonal and spiritual/religious issues facing someone with this disease. First, we describe a contextual model of factors affecting how one lives with sickle cell disease. Next, given that persons with SCD, like most people, are embedded in social and spiritual/religious contexts (that involve interactions with various relationship partners, including family members, friends, intimates, acquaintances, classmates, co-workers, health professionals, members of a religious congregation, and God), we wanted to examine how social interactions and spiritual/religious issues affect how someone copes with SCD. In particular, we hoped to identify what are significant social and spiritual/religious challenges affecting someone with SCD and with whom persons with SCD are having these issues. We believe that identifying major social and spiritual/religious issues related to living with SCD may be a useful first step in suggesting possible interventions to improve the quality of life as well as health outcomes for SCD patients.

A Contextual Model of Coping with Sickle Cell Disease

Health psychologists11 have proposed a conceptual model to identify the different factors (so-called "contexts") that influence how someone copes with any disease, including sickle cell disease. The contextual model focuses on the idea that there are reciprocal and interdependent relationships among the biological/medical, sociocultural, developmental and social/interpersonal factors that affect how one copes with a disease. We adapted this contextual model to describe how different contexts affect coping with SCD. After describing the relevance of the contextual model for how individuals deal with SCD, we examine, based on interviews with SCD patients, how social and spiritual/religious factors are related to living with sickle cell disease.

The biological/medical context focuses on sickle cell disease per se, including the genetic mechanisms underlying SCD, the acute painful episodes that are caused by vaso-occlusion by sickled red blood cells, and the numerous physical symptoms and complications that may be associated with this disease. The biological/medical context also includes how medical treatments and interventions affect living with sickle cell disease. Besides the biological/medical context, there are cultural, social, and spiritual/religious contexts that influence how one copes with this disease. The sociocultural context (especially culture and social class) focuses on the role of cultural attitudes about a particular group (based on ethnicity, race, social class), diseases and disabilities as well as geography, economic status, and access to medical care in communities in coping with SCD. For instance, given that patients with sickle cell disease in the United States are disproportionately likely to be African American, racial attitudes and stereotypes may, in part, influence attitudes about someone with sickle cell disease among health providers and the general public.12,13,14 Economic factors such as social class and family income may also influence how people view someone with sickle cell disease that, in turn, affects the kind of care available to SCD patients. The interpersonal context focuses on the social challenges affecting someone with sickle cell disease. Anyone with a chronic disease (including sickle cell disease patients) is embedded in social interactions with family members (including parents, siblings, aunts and uncles, grandparents), peers at work and in school, friends, intimate and dating partners, teachers, supervisors at work, one’s own children, and health professionals. These social interactions may be more or less supportive which, in turn, can impact on how well one copes with sickle cell disease.
Under stress, many individuals want to talk with others about their difficulties. Hence, individuals with sickle cell disease must also decide with whom to talk about sickle-cell disease-related issues. Many people living with a chronic disease (including sickle cell disease) also endorse a belief in God and in the power of prayer in coping, emphasizing the importance of a spiritual/religious context in living with sickle cell disease. Given the possible role of spirituality and religion in coping with sickle cell disease, it is important to understand how they affect coping with this disease.

Building on a contextual model of SCD, we focus on the social/interpersonal and spiritual/religious contexts involved in living with sickle cell disease.

Social/Interpersonal and Spiritual/Religious Challenges Facing Persons with Sickle Cell Disease

There is an extensive literature on the social/interpersonal issues (including helpful and unhelpful forms of social interaction, stereotyping, and stigmatization) related to living with chronic diseases. Numerous studies also exist on how spirituality and religion are related to coping with long-term diseases. Despite the extensive research on social/interpersonal and spiritual/religious contexts that affect coping with various health problems, there is a limited literature on what social and spiritual/religious factors are associated with SCD coping. Our interview study provides preliminary evidence about the types of interpersonal and spiritual/religious factors that are related to coping with sickle cell disease, including helpful and unhelpful social interactions with others, SCD-related stereotyping, participating in organized religious activities, types of prayer, and faith in God.

What types of social responses from others are perceived as helpful or unhelpful in coping with SCD? Also, who (in terms of type of a relationship partner) is identified as helpful and/or unhelpful in coping with sickle cell disease-related problems? There is an extensive literature indicating that social support may be beneficial in coping with stressful life events (including living with chronic diseases). However, someone under distress may not necessarily perceive that members of a social network are behaving in a helpful manner. In fact, previous research indicates that people make a distinction between helpful and unhelpful forms of social responses and that they may be differentially beneficial in dealing with disease-related stressors. Helpful forms of social responses may mitigate distress in coping with a disease, whereas unhelpful behaviors by others may exacerbate distress. In understanding the role of social interactions in coping with sickle cell disease, we wanted to examine (based on our interviews with SCD patients) what types of social responses are perceived as helpful versus unhelpful in coping with sickle cell disease as well as “who” enacts these particular behaviors.

What are the stereotypes and expectations that people are perceived to have about someone with SCD? Also, based on self-reports of persons with sickle cell disease, who is identified as holding these particular beliefs? There are several studies focusing on stereotypes that are held about someone with SCD, especially in the context of health providers interacting with SCD patients in emergency rooms and in hospitals. For instance, medical professionals in emergency rooms may believe that someone who is asking for pain medications for relief from sickle cell pain is a drug addict rather than someone who is seeking pain relief with narcotics. Given the possible role of stereotypes in how someone copes with SCD, we asked participants (based on their personal experiences) what were the different stereotypes and beliefs/expectations that they experienced as well as “who” endorsed these beliefs.

How does someone with sickle cell disease use spirituality and religion in coping with this disease? In our interviews, participants provided information about whether or not they regularly attended religious services and whether or not interacting with co-religionists was perceived as helpful in coping with sickle cell disease. Also, the participants provided information about whether or not they prayed and about the content of their prayers and communication with God. Prior research indicates that prayer and religious activities are important in coping with chronic health problems among African Americans. All the participants in our interview study were African Americans. Hence, we expected that spirituality and religion might play a significant role in descriptions of how someone copes with SCD.

Method

Research Participants

The participants were 24 individuals with sickle cell disease who had been invited to participate in an interview study on “Social and Personal Effects of Living with Sickle
Cell Disease." All the participants were patients at a SCD clinic located in southeastern Virginia. There were 6 men and 18 women in the sample. The mean age of the participants was 33.58 years (standard deviation = 8.79). All participants were African Americans. About 68% of the interviewees were either married or single with an intimate partner. Forty-eight percent were working either full- or part-time; 41.7% were on disability. Most participants (83.4%) had completed high school or had some college education.

A majority of the interviewees (58.3%) had been diagnosed with sickle cell anemia (Hb SS), whereas a smaller number had been diagnosed with either sickle cell hemoglobin (Hb SC; 25%) or sickle beta thalassemia (12.5%). Many participants reported having specific types of physical complications and/or problems associated with sickle cell disease in the last 9 to 12 months, particularly vision- and chest-related problems. Participants, on the average, reported having 7.39 (SD = 6.85) sickle cell-related pain episodes in the last 12 months. They reported that the average intensity of the pain experience was 8.52 (SD = 2.53) on a scale from 0 (no pain) to 10 (pain as bad as it can be). Participants also reported visiting an emergency room 3.37 times (SD = 2.53), on the average, in the last 12 months. They were hospitalized, on the average, 2.28 times (SD = 2.75) in the last 12 months. See Table 1 for a summary of demographic information about the participants.

Participants were interviewed in private offices at a sickle cell disease clinic. The length of the interviews ranged from 45 to 90 minutes. The interviews focused on a variety of interpersonal and religious/spiritual issues associated with living with sickle cell disease, including experiences with social support (helpful and unhelpful social responses by others), stereotypes and beliefs held by others about sickle cell disease, participation in religious services, and the content of prayers and communication with God. The interviews were audiotaped and subsequently transcribed for coding. Each participant was reimbursed $15.00 for the interview.

Content Analyses of the Interviews

The data were derived from a content analysis of the interviews. We focused on what social behaviors enacted by others were perceived as helpful or unhelpful, what were the stereotypes and beliefs about sickle cell disease that interviewees identified as being held by others, and what forms of religious/spiritual activities were used in coping with sickle cell disease (including belief in God, attendance at religious services, and communication/prayer with God).

Two doctoral students in clinical psychology (initial coders), in consultation with the first author, took the lead role in constructing the coding categories. First, each coder independently read 10 interviews and identified coding categories for helpful and unhelpful social responses by others, stereotypes/beliefs held by others about sickle cell disease, and forms of religious/spiritual activities used in coping with this disease. The two coders (along with the first author) met to compare and arrive at an agreement about the initial coding categories. The utility of the initial coding was then checked by having the initial coders read and code an additional eight transcripts; then the coders met again to check the utility of the initial coding scheme. After revisions, the revised coding categories for helpful/unhelpful acts and stereotypes/expectations were used to code the last six transcripts. Because one interviewee was unable to complete the part of the interview about spirituality and religion, the final coding of spirituality/religion was based on the coding of five transcripts. The coders met one last time with the first author to reach consensus on what would be the final categories for coding helpful/unhelpful social responses, types of stereotypes/beliefs about sickle cell disease, and forms of spiritual/religious coping. The coders’ construction of categories for helpful/unhelpful social acts and religious/spiritual activities were influenced, in part, by prior qualitative research on social support and religion/spirituality among persons with HIV.

For the actual data analyses the first author used the final coding categories to code all the transcripts. To assess coding reliabilities, another person (a psychology graduate student) independently coded twelve randomly selected interviews based on the coding schemes for helpful/unhelpful social responses, stereotypes/beliefs about sickle cell disease, and forms of spiritual/religious coping. The percentage of agreement in coding was high, exceeding 90% agreement for most of the scoring categories.

Participants’ Descriptions of Helpful Versus Unhelpful Behaviors As Well As Who Enacted Them

Interviewees were first asked to recall specific incidents when someone was helpful or unhelpful in
assisting them to cope with sickle cell disease. Interviewees were also asked to describe who enacted these helpful and unhelpful behaviors. Based on interviewees' descriptions, initial coders distinguished six types of helpful and seven types of unhelpful social behaviors. The primary judge used these categories to code what were identified as 120 helpful acts and 87 unhelpful acts enacted by specific others as mentioned in the interviews. See Table 2. The helpful categories included:

1. **Instrumental/tangible support** (36 of 120 helpful responses). Instrumental/tangible support means that another person does something concrete that is helpful in dealing with sickle cell disease-related issues. For instance, someone might provide assistance in getting the person with sickle cell disease to an emergency room or she/he might give financial assistance to help pay for medical bills. For instance, an interviewee said: “My wife takes me to my medical appointments.” Another interviewee said: “Sometimes if I get sick and I’m having a crisis in my legs and I can’t walk, and I have to go to the emergency room, my godfather is there to carry me.”

2. **Providing medical care/medical assistance** (29 of 120 helpful responses). This category means that someone (for instance, a medical professional or another person in one’s social network) either provided medical care directly or provided medical assistance to the person with sickle cell disease. For instance, an interviewee described how medical staff at a SCD clinic provided assistance with pain relief: “When I am sick or I have an exacerbation that doesn’t require hospitalization . . . . If I call my health team and I need pain medicine, I’m always able to get it right away. I don’t have to wait.” Another interviewee described how his father contributed to his medical care during experiences of SCD pain: “I live by myself, so there have been times when I have to call my father over because I’m in a lot of pain. He’ll come over, he’ll have medications, a heating pad, he will rub my body. Like when my legs hurt most he’ll rub them.”

3. **Emotional support** (25 of 120 helpful responses). Emotional support means that another person’s behavior was perceived as conveying empathy and concern. For instance, one interviewee reported how “My mother will call and check on me, asking how I am doing.” Another interviewee said example: “I was feeling sluggish and my son said, ‘Daddy, I’m going to pray for you.’”

4. **Informational support** (15 of 120 helpful responses). Informational support means that another person provides information, advice, or suggestions that are perceived as helpful in dealing with sickle cell disease-related issues. For instance, an interviewee described how “[Another sickle cell patient] explained to me what medications would be helpful because, honestly, I didn’t know. In the past, whenever I would go to the doctor, I never asked what is this for, what will this help me with.”

5. **Easing or sharing responsibilities at home** (9 of 120 responses). This category focuses on how another person does something tangible that reduces the household and/or domestic burdens of the person with sickle cell disease. For instance, an interviewee said: “My husband does things that are helpful because it takes a lot of pressure off of me. I don’t have to worry about the kids. I don’t have to worry about the household or anything like that. I can just focus on trying to get better or trying to rest, hoping that the crisis will pass and it won’t develop with me going to the hospital.”

6. **Easing/or sharing responsibilities at work or school** (6 of 120 helpful responses). This category focuses on how someone does things at work or school that eases the SCD person’s work- or school-related burdens. For instance, an interviewee (a college student) gave the following illustration: “Every single time she [another student in an Italian language class] didn’t see me in class, I would get a text message no later than 11:30 that day (describing what was done that day, what was on the test or what the class notes were on). I can’t say if she knows what sickle cell disease is or is not. However, she just sees that this is a class that I usually wouldn’t miss for the world and if I miss class it must be something going on.”

We should note (see Table 2) that mothers, other relatives (e.g., siblings, aunts, uncles, grandparents), intimate partners and physicians/nurses were frequently mentioned as sources of social support. Mothers, in particular, were often mentioned as providing emotional and instrumental support as well as some form of medical assistance. Physicians/nurses were mentioned most often in providing medical care/medical assistance.

The unhelpful categories of social behavior included:
1. **Erroneous beliefs and/or lack of knowledge about the biology/medical facts of SCD** (29 of 87 unhelpful responses). Many participants perceived that it was unhelpful when someone expressed erroneous facts about the biology and/or medical nature of sickle cell disease. For instance, an interviewee provided the following illustration: “I had a nurse tell me one time in a general hospital . . . she was my nurse for the night. She came in the door. I was having a real bad crisis. She asked me a stupid question of ‘how long have you had sickle cell disease?’ If she didn’t even know that you are born with sickle cell disease, then she couldn’t help me out.” Another interviewee described how other people felt that sickle cell disease was contagious. She said: “When I was young, back in grade school, other kids didn’t understand about SCD. I came to school but then I would get sick. When we had recess time and we had to go out to play, nobody wanted to play with me because they thought I was contagious. They would call me the sick girl, or don’t play with her . . . she has sickle cell disease. But they didn’t understand what sickle cell disease was at that time, so nobody wanted to play with me or to be my friend back then.”

2. **Lack of sympathy and/or empathy** (20 of 87 unhelpful responses). Someone displayed a lack of concern and/or empathy for the person with sickle cell disease. An interviewee gave the following example illustrating a lack of empathy: “This last time I had to go to the hospital, she [my wife] was sitting there, sulking with a long face. And I’m like, ‘what’s wrong, what’s going on.’ ‘You always have to go to the hospital. I’m sick of it.’ I felt like she was being really insensitive.”

3. **Other people minimized or denied the pain of the person with SCD** (16 of 87 unhelpful responses). Participants mentioned that other people may have minimized the SCD person’s experiences with pain and/or they believed he/she was faking about a pain episode. For instance, an interviewee described how physicians and her mother minimized her SCD pain: “I’ve had people [physicians] tell my mother [when I was younger], ‘She’s not in pain, she’s faking.’ I’ve always felt like, when I have to deal with my mom, she always feels like I was a liar, because that’s what the doctor would tell her. ‘But she can’t be in pain, she doesn’t have a fever. There’s no infection nowhere, she doesn’t have a fever; she can’t be in pain.’”

4. **Being described as a “drug seeker”** (13 of 87 unhelpful responses). Some participants said that it was unhelpful when someone perceived him and/or others with sickle cell disease as seeking narcotics for a “high” instead of pain management. An interviewee gave the following example: “[S]ome people will treat me like I’m just there [at the emergency room] trying to get pain medicine and using my disease as a crutch.” Another interviewee said: “I’ve been in a hospital and I’ve suffered in pain for hours because the doctors feel like all you want is pain medication. They feel like you’re addicted to the pain medicine.”

5. **Others did not ease up on school- or work-related responsibilities** (5 of 87 unhelpful responses). This category includes instances when someone did not provide assistance or relieve the SCD person’s work or school responsibilities when she or he had sickle cell disease-related health problems. For instance, an interviewee (a college student) described the following incident: “It was my freshman year of college. I had been hospitalized and I missed an exam. The professor actually made me take the exam one day after being in the hospital. I hadn’t studied for the exam and I had missed lectures. His only option to me was either I take the exam or I have to medically withdraw from the class. I didn’t want to withdraw from the class so I was forced to take the exam.”

6. **Someone did not ease up or share responsibilities when help was needed at home** (2 of 87 unhelpful responses). The person with SCD wanted someone (particularly a family member) to pitch in at home when she or he had a sickle cell disease-related problem. But they did not ease the domestic burdens of the person with SCD. For instance, an interviewee described how her mother would offer to care for her children when she was ill, though the mother did not follow through on this offer to help. “I know several times I’ve been sick and I needed my mother or my sister to get the kids before I even went to the hospital. My mother would say that my sister doesn’t have to come to get the kids because she is coming. Then my mother wouldn’t show up and my sister wouldn’t know about it.”

7. **Parent wasn’t helpful in managing or living with the disease while growing up** (2 of 87 unhelpful responses). An interviewee described how she was told to lie to other children while growing up about sickle cell disease-related problems. Concealing SCD, in turn, caused
her to feel ashamed about the disease. “Instead of saying I was out of school because of sickle cell or a crisis, my mother would say, ‘Oh, tell them that you hurt your arm, or you had a cold, or you had something.’ So, for the longest time, I became ashamed of my illness. As I started to get older, I realized that it’s not my fault. It’s nothing I did.”

Another interviewee recounted how her mother never informed her when she was growing up that she had sickle cell disease. “I was always sick and out of school, not feeling well, having pains in different places, not being able to dress up for physical education. Teachers thought that I didn’t want to do anything. Being sick and not even knowing that I had sickle cell; it went on for a long time. I found out [about having sickle cell disease] when I was 14 years old. I’ve had a rough life and my mother didn’t even tell me I had sickle cell. She knew when I was a baby.”

We should note (see Table 2) that physicians/nurses and emergency room staff were most frequently mentioned as sources of unhelpful behaviors, especially when they accused someone with sickle cell disease of being a drug seeker, when they minimized the interviewees’ sickle cell-related pain, and when they were perceived as saying something erroneous about sickle cell disease. Mothers were also frequently mentioned as unhelpful, especially if they displayed a lack of empathy or concern for the interviewees’ sickle cell disease-related health problems.

Participants’ Descriptions of Stereotypes and Beliefs/Expectations that People Have about Sickle Cell Disease and Who They Identify as Holding These Views

Interviewees were asked if they felt that other people had stereotypes or expectations about them because they had sickle cell disease. They were also asked who held these beliefs. Based on interviewees’ descriptions, the initial coders distinguished seven categories of stereotypes and beliefs/expectations about sickle cell disease. These seven categories were used by the primary judge to code 104 stereotypes/expectations held by specific others as mentioned in the transcripts. See Table 3. These categories stereotypes/expectations dealing with SCD included:

1. Beliefs that behavior and life choices need to be and/or should be restricted by sickle cell disease (27 of 104 responses). Many interviewees described how others expected that their life choices and activities should be restricted due to sickle cell disease. For instance, an interviewee described how “I do come across some people who want to baby me. They say, ‘Are you okay, can you do that, are you able to lift that?’ Or if we are going to go swimming or something like that they will ask, ‘Are you able to go swimming?’” Another interviewee said: “There are some people I know but don’t really know that well and they don’t know me that well. They’ll try to play it like, ‘Oh, I’m going to help you because I’m a good person and you’re a little weak.’”

A female interviewee described two instances where family members placed restrictions on her aspirations because of sickle cell disease. She said, “Growing up, my grandmother practically raised me. I wanted to go away for college. My grandmother said, ‘No, you have sickle cell disease. If you get sick, no one is going to be there.’ I have had chances to go to New York and do modeling. But my father said, ‘No, you have sickle cell disease. If you get sick, what are you going to do?’ So I felt like I was limited to just staying here in Virginia and settling for whatever.”

2. People with sickle cell disease are perceived to be drug addicts (20 of 104 responses). Interviewees gave many descriptions of being stereotyped as a drug addict, especially when pain medications were needed to cope with sickle cell pain episodes. An interviewee said: “Some people think that you are on drugs.” Another interviewee said: “The stereotype that I hate is that all sickle cell patients are drug seekers.” Another interviewee described how people believe “That we [individuals with sickle cell disease] are all junkies. We are all drug addicts.”

3. Erroneous beliefs and/or factual misunderstandings about sickle cell disease and its treatment (18 of 104 responses). This category includes inaccurate beliefs about sickle cell disease and medical treatments for this disease. For instance, a male patient described how he used an analgesic patch to manage sickle cell pain. A friend misunderstood the purpose of the patch: “So . . . I’ve had my best friend for fourteen years. When I first started taking the patch, he said, ‘What’s that, your birth control patch or your nicotine patch? He was just making a joke. But it is a stereotype; so I take it in stride.” A female interview described being stereotyped as having a contagious disease; this erroneous belief about sickle cell disease was mentioned by several interviewees. She said,
“When I was in the fifth grade, I was real quiet and I kept to myself. There was this one particular girl. I remember, clear as day, like it happened yesterday. We were on the swings and she started saying to anybody that tried to play with me, ‘Get away from her, she has sickle cell disease and you can catch it.’ That went on for two weeks, and it tore me up.”

Another interviewee described how emergency room staff didn’t believe he had sickle cell disease-related pain because he didn’t look or act sick. “They [medical staff in emergency rooms] stereotype all of us. You’re faking, you are not sick because you’re not bailing.” Another interviewer said, “I had one experience when I had to go to the ER. The nurses were applauding me because I didn’t want morphine or Demerol. But one nurse said, ‘Well, you’re not like the rest of the people with sickle cell disease. They just come here and fake for drugs.’”

6. Stereotypes and negative beliefs about the physical appearance of someone with sickle cell disease (8 of 104 responses). This category describes stereotypes and negative beliefs attributed to someone with sickle cell disease because of their physical appearance. For instance, an interviewee described how the color of his eyes [associated with jaundice] elicited negative reactions and stereotyping by others. “Sometimes stereotypes can upset you. When people look at me and see my eyes, yellow, or they look sometimes green, and a kid said, ‘He’s a monster.’” Another interviewee gave a related description of how the jaundiced color of her eyes elicited stereotyping, “I’ve had adults and children . . . and maybe some physicians and nurses, because my eyes are jaundiced, the stereotype is that you’re smoking some type of drug.”

A female interviewee gave the following example of how she was told that patients with sickle cell disease are small in stature: “I went to a doctor, one of the specialists, when I was younger. He just looked at me and said, ‘She doesn’t have it [SCD]. She doesn’t fit the profile for someone with sickle cell disease.’ The profile for sickle cell disease is that you are small and sickly looking. I am plump and juicy. I’m not by any means small. I don’t fit the stereotype.” A male interviewee reported a related stereotype about physical appearance and sickle cell disease. “I’ve actually had people in an emergency room ask several times, ‘Are you sure you have sickle cell? And they ask questions to try to trip me up. They ask that because of my appearance. You know a normal sickle cell patient is supposed to be very thin, yellow eyes, things like that. Me . . . I’m 250 pounds plus.”

7. Stereotypes and negative beliefs about the behavior of someone with sickle cell disease (4 of 104 responses). This category focuses on stereotypes about the allegedly difficult and/or bad behavior of people with sickle cell disease. An interviewee gave the following description of this stereotype: “A lot of people say that people with sickle cell disease are very mean when they have their crisis. I would say, probably so, because they’re in a lot of pain.” Another interviewee said he had heard physicians and nurses at various hospitals refer to patients with sickle cell disease as “We’re lazy, we all collect social security, we don’t want to do anything for ourselves but lay around and take drugs.” Another interviewee described
how emergency room staff perceived sickle cell disease patients as “nasty.” She said: “The stereotype is that they [sickle cell patients] are just nasty. They are the nastiest people. They have an attitude problem.” This stereotype was described by the interviewee in the context of how sickle cell patients, waiting for treatment, may be asked to answer “a thousand and one questions” before there is any attempt to control the sickle cell disease pain.

Overall, the most frequently cited stereotype (that is, daily activities and life choice of need to be restricted because of SCD) was attributed to several types of persons—including acquaintances/other people, other relatives, fathers, and to friends. In addition, interviewees cited emergency room staff most frequently for stereotyping someone with sickle cell disease as “drug seekers.” Interviewees also cited acquaintances/other people most frequently as holding erroneous beliefs about sickle cell disease, including the belief that the disease was contagious and it could be transmitted by casual contact.

Participants’ Descriptions of Spirituality and Religion in Coping with Sickle Cell Disease

We asked interviewees if spirituality and/or religion played any role in coping with sickle cell disease. Interviewees were asked to describe how this occurs (e.g., belief/faith in God, prayer, church attendance). This information was obtained from 23 of the 24 interviewees. One person was unable to complete the entire interview; no information was obtained from this person about religion/spirituality.

Initial coders constructed three categories to describe if faith in God, attendance at religious services, and support from co-religionists/clergy played a role in coping with SCD. They also identified several categories representing types of prayer and/or communication with God based on the interviewees’ descriptions. The primary judge used these categories to code how many persons (out of the 23 interviewees who completed this part of the interview) mentioned faith in God, regular attendance at religious services, and support from co-religionists. The primary judge also coded how many interviewees out of the 23 interviewed about religion/spirituality mentioned using a particular type of prayer and/or communication with God. See Table 4 for a summary of these results.

Faith in God and Church-Related Activities

1. Faith in God (22 out 23 interviewees indicated that faith in God played a role in coping with SCD). Faith in God was described in different ways. For instance, an interviewee described how “With sickle cell disease, we are not in this alone. Even though sometimes family might forsake us, friends might not understand, God is all-knowing. God has all-knowing power in his hand. And that is your source of life.” Another interviewee said, “I believe in God, Jesus Christ, and I know that he’s there with me.” Another interviewee said, “It [religion/spirituality] has a pretty tremendous role in coping with sickle cell disease because if it wasn’t for my belief in God and my faith then I would not be here. So, church and my belief in God and my religion are first and I give all the credit to Him.”

2. Regular attendance at religious services (14 of 23 interviewees indicated that they attended religious services regularly).

3. Social support provided by other members of one’s religious organization and/or clergy (13 of 23 interviewees indicated that they had received support from co-religionists and/or from clergy). Interviewees described different forms of social support that was provided by co-religionists and/or clergy. This support might be spiritual and/or instrumental. For instance, an interviewee indicated how “They [church members] knew about me having sickle cell disease. When I would get sick they all would come to the hospital, visit with me, and pray with me. Anything that I needed they would do it.” Another interviewee said, “There is a Community Prayer Band. It’s a lot of ladies from the community who get together and they go to the hospitals. They visited me plenty of times in the hospitals. If I was at home and I missed church because I was sick, they would come to the home and prayer. That has made a difference.” Another interviewee described how she was participating in a 40 day fast as part of her religious activities, but her minister helped relieve her of this obligation that might have caused health complications. “When we did the 40 day fast, I got really sick because I didn’t compromise. I didn’t take my medications, I didn’t eat food, and so I was more eating the word of God versus anything of nutrition. So, with that I had to go to my pastor and say, ‘Look, I can’t do this.’ And the next time that we met [at church], he started giving disclaimers. ‘If you have diabetes, if you know you have sickle cell disease, God knows your heart and he’s not going to cast you down into the pits of hell because you had to drink water and take your medications.”
Types of Prayer and Communication with God about Sickle Cell Disease

Twenty two of the 23 interviewees (who were asked about religion/spirituality) described some form of prayer and/or communication with God. These included:

1. Praying for relief from pain or to avoid sickle cell-related pain and medical problems (19 of 23 interviewees reported this type of prayer). For instance, an interviewee described how prayers served to provide relief from sickle cell disease-related pain. “My prayers to God about the disease are for me to not be in so much pain. And when I pray and I’m not in that much pain, I feel like it’s all because of my prayer.” Another interviewee said, “I don’t pray to take the illness away. I don’t say it’s not possible. But I always pray to let me get through it, at least that first part, the painful episodes.” Another interviewee said how she prayed that God would help medical staff to deal with her pain: “When I’m in pain, dear God, dear Jesus, dear God. I just keep repeating His name, because what else can you say? Technically, He’s the only one that can help you. The doctors are there but they can do only what He allows them to do in the first place. I pray, ‘God, help them to do the things that you want them to do that will benefit me.”

2. Praying to God for strength dealing with sickle cell disease (6 of 23 interviews reported this type of prayer). For instance, an interviewee described how “I pray for strength to cope with it [sickle cell disease].” I’d pray that God would give me the strength to overcome this, not let it control me or run me,” Another interviewee described praying to God to keep focused in dealing with sickle cell disease. “I pray normally for guidance. It’s more of a mental ‘help me keep my mind open and clear of negative thought and to stay focused.’ Sometimes the pain [associated with sickle cell disease] can be extreme and you are praying. ‘Dear God, please stop the pain.’” But for the most part I’m a logical person and I just want to make sure that my head stays clear to where I can say ‘these are the steps I want to take to get better. It’s going to eventually happen, just keep me strong enough and don’t let me break down.”

3. Praying and communicating to God to express gratitude for being alive, thanks for relief from sickle cell disease-related health problem, or acceptance of a purpose in life in having sickle cell disease (6 of 23 interviewees reported this type of prayer). For instance, an interviewee said, “A lot of people with sickle cell disease do pretty bad, but I don’t think I’m as bad off as other people when I have a crisis. I normally thank God for that and just ask Him to continue to bless me and enable me to be here.” Another interviewee said, “To be honest I think I could have been dead a long time ago, but I am still here on earth. So, I know He [God] has a plan for me and I am just waiting on him to show me the way.”

4. Praying and/or communicating to God about “Why do I have sickle cell disease?” (5 of the 23 interviewees mentioned this form of prayer/communication). This category focused on interviewees’ questioning God about the purpose for their having sickle cell disease. For instance, an interviewee said, “At one point in time, I felt like I had a lot of issues to deal with in my relationship with God. I was going through so much and there was no answer. So, I was doing something that I was always taught not to do, and that is asking God, ‘why?’” Another interviewee expressed similar reasoning about “why me” in communicating with God. “I would talk to God trying to get a better understanding of why. Why do I have it [sickle cell disease] and what is the purpose of me having it and what am I to do?”

5. Praying in behalf of others (3 of the 23 interviewees mentioned this form of prayer). For instance, an interviewee said, “It may sound crazy. But when something is going on in our family and somebody is very sick or somebody is going through something, sometimes I’ll pray and ask God to let me take on their burden. I’ll go through a crisis for them not to go through what they are going through. I do that sometimes too because I know I can take that pain for a couple of days and it will go away. If that would help somebody else go through what they are going through, I don’t mind.”

6. Praying for oneself that is unrelated to sickle cell disease (3 of 23 interviewees mentioned this form of prayer). For instance, an interviewee described that he prayed to God for help in finding an intimate partner. “At some point, I am praying to God about send me a mate because I’m lonely down here.” Another interviewee said, “I pray to God all the time for everything and anything.”

7. Expressing anger (1 of 23 interviewees mentioned expressing anger to God for not looking after him during SCD pain episodes. He said, “I was brought up
in church and I was a faithful attender. I was so close to God and then I got really sick. A lot of times I have cursed God. I told Him he had forgotten me, that he must hate me. It’s not fair, You are not there with me. You don’t understand. I have fussed at God so many times. I’d be surprised if I get there and God says, ‘Your’re name is not in my book,’ because I was so evil to him.”

Discussion

The results of the interviews provide preliminary evidence about the social/interpersonal and religious/spiritual issues confronting individuals who have sickle cell disease. Interpersonally, someone with sickle cell disease may have access to socially supportive behaviors from others (including emotional, instrumental, informational, and assistance with medical care/medical treatment). However, other people’s behavior may also be unhelpful (especially when these unhelpful responses are perceived as lacking in understanding about the disease or in sympathy, minimizing the severity of SCD pain, and erroneously labeling SCD patients as drug seekers). These results highlight the importance of both helpful and unhelpful types of social interactions in the lives of individuals living with sickle cell disease. Helpful and unhelpful behaviors were attributed to a wide range of persons. In particular, helpful acts were most frequently attributed to mothers, physicians/nurses, other relatives, intimate partners, and friends. Helpful acts were infrequently attributable to emergency room staff. In contrast, unhelpful acts were most frequently attributed to physicians/nurses, mothers, and to emergency rooms staff. It is interesting that the same people—especially mothers and physicians/nurses—might be perceived as helpful and/or unhelpful, indicating the importance of both types of these interactions with specific others for SCD patients.

Given a long history of research documenting that positive and negative social interactions “represent relatively independent domains of experience” (p. 1106) and that they may differentially predict psychological distress and negative emotional well-being, future research should separately assess helpful and unhelpful reactions to people with sickle cell disease and, in turn, how these interactions might predict coping with sickle cell disease. For instance, previous research with HIV patients and older persons found that negative social interactions (e.g., failure to provide help, unsympathetic or insensitive behavior) have a greater effect on measures of psychological distress than positive social exchanges (e.g., instrumental support, emotional support). Given prior evidence for this negativity effect where “the harmful effects of negative exchanges outweigh the beneficial effects of positive exchanges” (p. 309) on mental health outcomes, research should examine how unhelpful social responses might have a more deleterious effect on coping with sickle cell disease relative to the benefits derived from helpful social responses. Also, research could examine possible mediators in the association between helpful and unhelpful forms of social interaction and health outcomes. For instance, others’ helpful versus unhelpful responses to one’s sickle cell disease-related difficulties could affect health outcomes via several routes—cognitive (e.g., affecting self-esteem and self-efficacy to deal with one’s health problems and life situation), emotional (vulnerability to anxiety, depression, and anger), and health-related behaviors (e.g., alcohol consumption, smoking cigarettes, eating fast foods, intake of fruit, vegetables, exercising, adherence to medical regimens).

The research also identified a wide range of stereotypes and beliefs that are held about sickle cell disease, according to the interviewees. Two frequently cited stereotypes include the belief attributed to others that there should be restrictions in the daily activities and life choice of someone with SCD and that patients with SCD are sickly and live a shorter life. One woman described how family and friends looked at her as if her situation was “hopeless.” Others noted how physicians had said (especially to the interviewees’ parents) that the interviewee would have a shortened life span. A major issue for most SCD patients likely focuses on understanding and coping with the medical, psychological, and social challenges posed by SCD (e.g., managing pain episodes, going to school, having a job, being in an intimate relationship, raising a family), in the context of distinguishing between real limitations posed by SCD versus one’s own and others’ expectations about what one can and cannot do.

Many interviewees also described being stereotyped as a “drug seeker” or someone who was lying or faking pain—especially by emergency room staff and/or physicians/nurses. This finding replicates the results of previous studies documenting that sickle cell disease patients risk being labeled as drug addicts or faking pain by medical staff when they utilize an emergency room to deal
with an acute painful episode caused by the vaso-occlusion of sickled red blood cells.14 Given that many SCD patients (including our interviewees) perceive that medical personnel mistakenly stereotype them as drug addicts who are faking pain, it is not surprising that SCD patients often prefer managing their disease (especially sickle cell related pain episodes) at home as opposed to using medical facilities.3 Some of the interviewees explicitly mentioned their preference for being patients at the Sickle Cell Clinic (where our interviews were conducted) as opposed to going to an ER. These patients felt that the SCD Clinic staff was especially knowledgeable about sickle cell disease (including its etiology and treatment) compared to medical personnel that they met at ERs. They also valued having an on-going relationship with the physicians and nurses at this SCD clinic instead of being “walk-ins” at an ER.

We did not directly ask interviewees (all of whom were African Americans) if and how racial factors might in some way affect others’ reactions to them. Nevertheless, four interviewees mentioned race as a factor in living with SCD. Three interviewees spontaneously mentioned that SCD is more likely to be a health problem for African Americans than for other racial/ethnic groups. One of these interviewees mentioned that “SCD is slipped under the rug because it’s the Black race that has it. It doesn’t run in the White race, so the government really feels like, to me that they don’t have to do anything about it.” Two other interviewees expressed the view that “other groups” (including Whites) have a lack of personal familiarity with sickle cell disease that, in turn, leads to a lack of understanding and a willingness to stereotype someone with SCD. One person said, “I feel like because it’s more of a Black disease people don’t watch with us and experience it. Because they can’t experience it [SCD] it’s easy to judge somebody with the disease.” A fourth interviewee was disconcerted that a Black woman who worked with her mother at an assisted living facility spoke badly about people with SCD. “We’re all dirty, we’re nasty. She talks about sickle cell patients really, really bad. And this is a Black woman.” It would be useful to examine possible racial differences in how Blacks and Whites (laypersons as well as health professionals) perceive someone with SCD, and how prior experiences being the target of racial discrimination might be associated with SCD patients’ reactions to the care that they receive in hospitals and ERs and to their overall psychological and physical health.35,36

Almost all the interviewees noted the importance of spirituality and/or organized religion in coping with SCD. Most of the interviewees also endorsed the importance of God in coping with SCD. Also, more than half of the interviewees reported that they regularly attended religious services and that clergy and/or church members had provided support in coping with SCD. These findings are consistent with other research documenting that many African Americans rely on God as a source of strength in dealing with distress and that they use attendance at religious services and prayer in coping with chronic health problems (including SCD).17,18,25 Nevertheless, at least one study reported mixed results about the role of church attendance and prayer/Bible study on relieving sickle cell-related pain.17 Future research should examine how the different aspects of spirituality/religion reported by our interviewees (including faith in God, church attendance, support provided by minister/church members, type of prayer/communication with God) might be related to coping with sickle cell disease as well as to psychological and physical well-being. Although only one interviewee mentioned that he had expressed angry feelings to God during pain episodes, research should also consider how the perception of nonsupport from God may affect coping with SCD.

Conclusions

Our results are preliminary, but it suggests several conclusions. First, individuals with SCD make a distinction between helpful and unhelpful social responses enacted by others as they cope with this disease. Given that persons with SCD make a distinction between positive and negative social exchanges, it is important to understand how these types of social exchanges might be related to psychological and physical well-being in coping with SCD.

Second, we have confirmed previous research documenting that sickle cell disease patients perceive that stereotypes about the disease (e.g., individuals with SCD are addicted to narcotics and they fake pain) are widely held by medical professionals—especially by ER staff. On the other hand, our interviews document that SCD patients also perceive that their family, friends, and acquaintances hold stereotypes and expectations, especially that their behavior and life choices should be restricted. Some participants felt that they were overprotected in childhood and adolescence and that family and friends continue to be
overprotective. Future research needs to examine how individuals with SCD move beyond the protective behaviors enacted by care providers in childhood and adolescence to finding a balance between what one can and cannot do in adulthood (living a normal life to the degree possible while also coping with unpredictable pain episodes, developing relationships based on trust and mutual respect with health providers who are knowledgeable about SCD, and taking care of one’s health needs on a daily basis).

Third, our results indicate the important role of spirituality and religion in coping with SCD. Although participation in a religious organization played an important role in coping with SCD for many interviewees, the results also documented the significance of private prayer and communication with God in dealing with this disease. Prayer and talking with God may be valuable assets for many spiritually-oriented individuals in seeking relief from sickle cell disease-related pain and in making sense of being afflicted with this disease.

Finally, we should note that a contextual analysis of sickle cell disease was useful in “shining a light” on a variety of social/interpersonal and spiritual/religious factors affecting how someone lives with SCD. Besides the biological/medical factors that influence living with sickle cell disease, someone with sickle cell disease is also embedded in a variety of social, cultural, and spiritual/religious contexts that will affect their physical and psychological well-being.

**Policy Implications**

Much of psychological research on SCD (with important exceptions) has focused on the role of individual-based factors (e.g., daily stress, cognitive and behavioral strategies of coping, self-efficacy in coping with SCD, and self-hypnosis) in pain management and promoting the quality of life for SCD patients. Nevertheless, our research indicates how cultural, social, and spiritual/religious factors should be taken into account to design interventions to improve the quality of life (including health outcomes) of people with SCD. For instance, interviewees often mentioned how family members, intimate partners, acquaintances, and health professionals misunderstood the nature of SCD. A possible intervention would be to educate other people (who are embedded in a patient’s social network) about SCD-related issues. In this context, it is useful to mention a video-intervention that Haywood and colleagues have created to improve clinicians’ attitudes about SCD patients. This educational intervention (based on a 8-minute video involving a hematologist and three adult SCD patients who talk about issues SCD patients face in seeking treatment for pain), compared to a control condition, increased clinicians’ positive attitudes about SCD patients (e.g., “Are satisfying to take care of”) and reduced negative attitudes about SCD patients (e.g., “Are drug see-seeking when they come to the hospital”). This simple intervention was designed to improve clinicians’ attitudes about SCD patients, but it might also be useful to show to anyone in SCD patients’ social network so significant others can better understand medical challenges confronting someone with SCD.

Along with the importance of cultural/social factors involved in living with SCD, the research suggests how religious communities (as support providers) and a spiritual mindset may be useful in assisting individuals to cope with SCD. A reliance on “Faith in God” by itself is insufficient in coping with SCD, but spiritual/religious interventions (e.g., prayer and communications with God, social support from co-religionists and clergy) may assist SCD patients (who are spiritually oriented) to find solace and purpose in living with this disease and, in turn, to better take care of themselves in day-to-day living with SCD.
References


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<td>Heat, e.g., hot bath or heating pad</td>
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<td>66.7</td>
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Rest and stop all activity 17 70.8
Narcotic pain medication 20 83.3
Non-narcotic pain medication 13 54.2
I did not have pain during the last 12 months 1 4.2

What is your specific sickle cell disease?
Sickle cell anemia (Hb SS) 14 58.3
Sickle cell hemoglobin (Hb SC) 6 25.0
Sickle beta thalassemia 3 12.5
Don’t know 1 4.2

Have you had any of the following physical complications or problems in the last 9 to 12 months?
Leg sores or leg ulcers 4 16.7
Kidney-related problems 3 12.5
Bone damage 4 16.7
Vision problems 13 54.2
Chest related problems 10 41.7
Seizures 1 4.2
Strokes 0 0
Painful long-lasting erections (that is, priapism) 1 4.2

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Table 2

*Frequency of Helpful and Unhelpful Social Behaviors Enacted by Specific Others as Mentioned by Interviewees*

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<th>Mother</th>
<th>Other Relative</th>
<th>Intimate Partner</th>
<th>Friend</th>
<th>Neighbor</th>
<th>Co-worker/Fellow student</th>
<th>Supervisor</th>
<th>Teacher</th>
<th>Physician/Nurse</th>
<th>Emergency Room Staff</th>
<th>Acquaintance/Other people</th>
<th>Own Children</th>
<th>Someone with SCD</th>
<th>Church Member/Minister</th>
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<tr>
<td>Eased/Shared responsibilities at work/school</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>24</td>
<td>14</td>
<td>13</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>17</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>120</td>
</tr>
</tbody>
</table>

Types of Unhelpful Responses by Others:

<table>
<thead>
<tr>
<th>Types of Unhelpful Responses by Others:</th>
<th>Father</th>
<th>Mother</th>
<th>Other Relative</th>
<th>Intimate Partner</th>
<th>Friend</th>
<th>Neighbor</th>
<th>Co-worker/Fellow student</th>
<th>Supervisor</th>
<th>Teacher</th>
<th>Physician/Nurse</th>
<th>Emergency Room Staff</th>
<th>Acquaintance/Other people</th>
<th>Own Children</th>
<th>Someone with SCD</th>
<th>Church Member/Minister</th>
<th>God</th>
<th>Counselor</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Factual misunderstanding about SCD</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Lack of sympathy/Empathy</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Minimized or denied the pain of the individual</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Perceived individual as a drug seeker</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Did not ease/Share responsibilities at work/school</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Did not ease/Share responsibilities at home</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Didn’t help her/him manage the disease growing up</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>16</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>19</td>
<td>15</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>87</td>
</tr>
</tbody>
</table>
Table 3

*Frequency of Stereotypes and Expectations Held by Specific Others about SCD According to Interviewees*

<table>
<thead>
<tr>
<th>Stereotypes and expectations about sickle cell disease</th>
<th>Father</th>
<th>Mother</th>
<th>Other Relatives</th>
<th>Intimate Partners</th>
<th>Co-worker</th>
<th>Supervisor</th>
<th>Teacher</th>
<th>Physicians/Nurses</th>
<th>Emergency Room Staff</th>
<th>Acquaintance/other people</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavior and life choices should be restricted</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Considered a narcotics drug seeker</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>11</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Erroneous beliefs about SCD</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Believed to have short life span, sickly, or weak</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Considered a liar/faking pain</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Negative beliefs associated with appearance</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Negative beliefs associated with behavior</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>1</td>
<td>2</td>
<td>13</td>
<td>22</td>
<td>34</td>
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</tbody>
</table>
Table 4

*Number of Interviewees Who Mentioned a Particular Type of Spirituality/Religious Activity in Coping with Sickle Cell Disease*

<table>
<thead>
<tr>
<th>Type of Spirituality/Religious Activity</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faith in God</td>
<td>22</td>
</tr>
<tr>
<td>Attends religious services regularly</td>
<td>14</td>
</tr>
<tr>
<td>Support provided by minister and/or church members</td>
<td>13</td>
</tr>
<tr>
<td>Type of prayer/communication with God:</td>
<td></td>
</tr>
<tr>
<td>For relief from pain</td>
<td>19</td>
</tr>
<tr>
<td>For strength dealing with SCD</td>
<td>6</td>
</tr>
<tr>
<td>For thanks in coping with SCD/gratitude for a purpose in life</td>
<td>6</td>
</tr>
<tr>
<td>Why me?</td>
<td>5</td>
</tr>
<tr>
<td>For others</td>
<td>3</td>
</tr>
<tr>
<td>For non SCD-related issues</td>
<td>3</td>
</tr>
<tr>
<td>Expressing anger</td>
<td>1</td>
</tr>
</tbody>
</table>

*Note*: n equals the number of participants who mentioned faith in God, regular attendance at religious services, support provided by co-religionists in coping with SCD, or a particular type of prayer/communication with God. One interviewee did not complete the last part of the interview focusing on spirituality/religious activity. Hence, there were 23 interviewees who provided information for this part of the interview.
Increasing Initiation of Hydroxyurea in Pediatric Patients with Sickle Cell Disease

Authors: Amy Sobota, MD, MPH; Ebaa Jastaniah, MD; Philippa Sprinz, MD, MSc; Patricia Kavanagh, MD; William G. Adams, MD; James Moses, MD, MPH

Affiliations: 1 Boston Medical Center Department of Pediatrics, 2 Boston University School of Medicine, 3 Tufts University School of Public Health

Abstract

Background: Despite the proven benefits of hydroxyurea (HU) in preventing complications of sickle cell disease (SCD), it remains underutilized.

Methods: A QI project to improve patients’ and parents’ knowledge of HU.

Results: Of 106 eligible patients, 31 (29%) were not on HU. After completing our interactive educational module, knowledge of HU increased from a mean of 1.56 to 2.78 (out of 3) correct questions and likelihood of initiating HU increased from a mean of 2.4 to a mean of 3.8 on a 5-point Likert scale. Of the 20 families who completed HU education 13 initiated treatment with HU.

Conclusion: This work showed that a targeted educational intervention can have significant impact on patients'/guardian's understanding of HU and likelihood to initiate treatment.

Introduction

Sickle cell disease (SCD) is an inherited red blood cell disorder characterized by anemia, vaso-occlusive pain episodes, multi-organ damage and early death, which affects approximately 100,000 people in the United States. Through advances in medical care, such as universal newborn screening for hemoglobinopathies, prophylactic antibiotics, and targeted immunizations, almost all children with SCD now survive to adulthood. However, SCD continues to be associated with severe complications that include stroke, acute chest syndrome, sepsis and pain episodes requiring frequent hospitalizations.

Hydroxyurea is the only medication approved by the FDA for treatment of SCD, and has been shown to reduce the frequency of vaso-occlusive painful episodes, acute chest syndrome, hospitalizations and mortality in adults. Similar results have been demonstrated in children.

Despite the proven effectiveness of HU in managing SCD, it remains underutilized. Documented barriers to the use of HU include the following: healthcare providers' awareness, patient knowledge/beliefs, physicians' concerns for patient compliance (taking medication, getting blood tests, and using contraception) and providers' perceptions of families' concerns over side effects.

The goals of our work were to improve the knowledge, beliefs and attitudes of patients and parents of children with SCD towards HU. We hypothesized that this improved knowledge and understanding would increase the acceptance of HU among patients with HbSS.

Methods

Participants: This quality improvement project was conducted in an academic pediatric hematology practice within an urban safety net hospital. The pediatric practice provides comprehensive care for approximately 180 patients ages birth to 21 with SCD. As of June 2012 patients in our clinic were deemed eligible for HU if they were between the ages of 1 and 21 years with a genotype of HbSS or HbSβthalassemia who were not receiving chronic blood transfusions. Eligible patients who were not on HU were identified through the hospital's SCD patient registry. These patients (or their parent/guardian) were called and invited to attend a clinic session to learn more about HU.

Intervention:

We held a focus group of patients or parents of patients on HU to guide the development of our intervention. Using Plan-Do-Study-Act cycles based on the Institute for Healthcare Improvement (IHI) model for improvement, we created and implemented an interactive educational module for patients and their families. The material included information about HU (including images showing the effect of HU on red blood cells and data from a recent study showing improved survival for patients treated with HU), a video created by the National Heart, Lung and Blood Institute and parents’ testimonials from the focus group.
We created and tested a brief (5-question) multiple-choice test to assess patients'/guardians' knowledge, beliefs and attitudes on HU. Three multiple choice questions with one correct answer were used to determine the patients'/guardians' understanding of the role of HU in SCD. A single question was asked for the likelihood that a patient/parent would initiate treatment with HU, scored on a 5-point Likert scale with 1 = 'very unlikely', 3= 'neutral' and 5= 'very likely'. The final question asked about barriers to the utilization of hydroxyurea and allowed multiple answers. Data was collected pre- and post-intervention.

The educational module was administered by clinic staff during specially scheduled HU educational visits. After completing the pretest assessment, a provider presented the educational module to the patient/guardian allowing an opportunity for discussion. The posttest assessment was then completed with the same series of questions and scoring. The total session lasted approximately 30 minutes.

This project was deemed exempt by the Boston University Institutional Review Board. Funding was provided by the Sickle Cell Disease and Newborn Screening Program of the Health Resources and Services Administration grant # U38 MC22215.

Results

Out of 181 active patients, 106 patients (58%) met our eligibility criteria for HU. Seventy-five (70%) were already on HU, leaving 31 (29%) patients eligible for, but not already taking HU. Twenty-one of these families accepted an offer to learn more about HU (5 were not already taking HU, leaving 31 (29%) patients eligible for, but met our eligibility criteria).  Seventy patients (38%) were prescribed HU.  Seventy-five (70%) were already on HU, leaving 31 (29%) patients eligible for, but not already taking HU. Twenty-one of these families accepted an offer to learn more about HU (5 were not already being on HU, leaving 31 (29%) patients eligible for, but not already taking HU. Twenty-one of these families accepted an offer to learn more about HU (5 were not already taking HU, leaving 31 (29%) patients eligible for, but met our eligibility criteria).  Seventy patients (38%) were prescribed HU.  Seventy-five (70%) were already on HU, leaving 31 (29%) patients eligible for, but not already taking HU. Twenty-one of these families accepted an offer to learn more about HU (5 were not already being on HU, leaving 31 (29%) patients eligible for, but not already taking HU. Twenty-one of these families accepted an offer to learn more about HU (5 were not already taking HU, leaving 31 (29%) patients eligible for, but met our eligibility criteria).  Seventy patients (38%) were prescribed HU.  Seventy-five (70%) were already on HU, leaving 31 (29%) patients eligible for, but not already taking HU. Twenty-one of these families accepted an offer to learn more about HU (5 were not already being on HU, leaving 31 (29%) patients eligible for, but not already taking HU. Twenty-one of these families accepted an offer to learn more about HU (5 were not already taking HU, leaving 31 (29%) patients eligible for, but met our eligibility criteria).  Seventy patients (38%) were prescribed HU.  Seventy-five (70%) were already on HU, leaving 31 (29%) patients eligible for, but not already taking HU. Twenty-one of these families accepted an offer to learn more about HU (5 were not already being on HU, leaving 31 (29%) patients eligible for, but not already taking HU. Twenty-one of these families accepted an offer to learn more about HU (5 were not already taking HU, leaving 31 (29%) patients eligible for, but met our eligibility criteria).  Seventy patients (38%) were prescribed HU.

During the initial 2 months of this QI project, 9 families completed the pre- and post-education assessments. The most common reasons cited for the patient not already being on HU was 'not sick enough' (n=4) and 'worry about side effects' (n=3). Other reasons included "do not have much information about it" and 'HbF is high". After completing the teaching module with a staff member, the patient/parent knowledge of HU increased from a mean of 1.56 to 2.78 (out of 3) correct questions and the likelihood of initiating HU increased from a mean of 2.4 to a mean of 3.8 on the 5-point Likert scale (figure 1). When asked if the information presented changed their thoughts about HU, families commented on the importance of learning that 'it is used for problems other than pain' and 'it helps live longer'.

At the end of the first 2-month period we stopped collecting pre- and post-test data due to time constraints and staffing. However we continued to reach out to families over the next 9 months and completed HU education with a total of twenty families. As of April 2014, 13 initiated treatment with HU, 5 were still considering it, and 2 declined (figure 2). Given this increase at the end of the project 79% of eligible patients were prescribed HU.

Discussion

Despite its demonstrated efficacy, HU is underutilized in the treatment of SCD. We undertook an internal quality improvement project to increase patient/guardian knowledge of HU with a goal to increase its acceptance among families, many of whom had previously declined to initiate treatment. By creating and implementing an interactive educational module we were able to increase acceptance of HU among families of patients with SCD. This led to an overall increase in the proportion of eligible patients in our clinic on HU from 58% to 79%.

Recent work suggests that patients with SCD suffer organ damage between or without acute complications. This makes HU an appropriate treatment even for patients who may not be overtly symptomatic. However classic criteria for instituting HU depend on frequent acute complications. This study demonstrates that patients and guardians of patients who had previously declined treatment with HU may have had done so because of a belief that their child was not 'sick enough'. However learning about the increased survival of patients on HU and that HU can have effects beyond reducing acute painful episodes was persuasive to many families.

The use of HU is complicated by many misperceptions and misbeliefs about the medication. This study showed that a targeted educational intervention can have an impact on patients'/guardians' understanding of HU.

Limitations of this work include a small sample size; however the barriers reported by our patients were similar to those in the literature, which makes it likely that our findings may be representative of SCD patients in general. We also did not address adherence, however different methods are necessary for such work.

Educational efforts targeting the preventative roles that HU plays in complications of SCD should be considered to increase utilization. Additional work to address underlying beliefs about the medication may be necessary, and ongoing educational efforts will be needed to improve adherence.

References:
References:


Acknowledgements: The authors wish to thank Delmaude Farrell and Nacy Brandao for their assistance with this work. Funding was provided by the Sickle Cell Disease and Newborn Screening Program of the Health Resources and Services Administration grant # U38 MC22215. We have no conflicts of interest to report.
Figure 1: Pre-test and post-test assessment of (A) patient/parent knowledge of HU and (B) patient/parent likelihood to initiate treatment with HU

Figure 1A: Patient/parent knowledge of hydroxyurea

Figure 1B: Patient/parent likelihood to initiate hydroxyurea
*due to special circumstances such as custody issues or plans to move out of the area
Redefining Chronic Hemolysis in Pediatric Sickle Cell Disease: Focus on Symptomatic Cholelithiasis

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Keywords: Sickle Cell Disease, Hemolysis, Gallstones

ABSTRACT
Background. Chronic hemolysis leads to increased bilirubin levels and subsequent gallstones in sickle cell disease (SCD). However, there are no published articles focusing on gallbladder pathology in pediatric SCD and its relationship to other complications. The goal of this retrospective case-control study was to compare the frequency of SCD-related complications in patients who underwent cholecystectomy compared to those who had not.

Results. Approximately 75% of cases were symptomatic, while 12% had frank cholecystitis. Children undergoing cholecystectomy had an increased frequency of stroke, greater likelihood of conditional or abnormal TCD, and increased pain admissions. Cases ages 3-6 years had increased SCD morbidities overall as compared to age-matched controls.

Conclusions. This study suggests that early gallbladder disease may be a marker of increased clinical severity.

INTRODUCTION
Nearly 100,000 Americans are affected with sickle cell disease (SCD), making it one of the most prevalent genetic disorders in the United States. Inherited in an autosomal recessive fashion, individuals with SCD can exhibit significant morbidity and mortality related to chronic hemolysis [1]. Whereas SCD was once seen as a disease where morbidity/mortality were directly related to vascular occlusion by red cell sickling alone, it is now thought that chronic hemolysis secondary to endothelial dysfunction and vasculopathy plays a significant role in the morbidity of this disease. Researchers have found that release of hemoglobin and arginase from red blood cells leads to impaired nitric oxide (NO) bioavailability, thereby resulting in increased oxidative stress, hemostatic activation, and acceleration in intravascular hemolysis [2]. SCD confers a state of NO resistance and, since NO is essential for endothelial cell regulation, decreased levels attenuate vascular response to NO-mediated stimuli [3]. Kato and colleagues have defined a phenotypic model of SCD that links certain clinical complications largely to blood viscosity and vaso-occlusion and other complications primarily to hemolysis-associated vasculopathy [4]. Pulmonary hypertension, chronic leg ulcerations, priapism, and stroke have been associated with decreased NO bioactivity and a more severe baseline hemolytic anemia whereas pain crises, acute chest syndrome (ACS), and avascular necrosis (AVN) seem to be more related to increased vasocclusion and a relatively higher hematocrit.

Chronic hemolysis with a resultant increase in bilirubin turnover leads to a high incidence of pigment gallstones. The onset of cholelithiasis can be as early as 2 to 4 years of age. Prevalence increases progressively with age, with reports of 17-33% of patients in the 2-18 year age range with gallstones [5]. Biliary sludge may be an antecedent of
gallstones. Studies in patients with SCD indicate that sludge is often found with stones, but sludge alone may or may not progress to stone formation. However, the follow-up period of such studies has been limited [5,6]. Further, studies of gallbladder function indicate that those with cholelithiasis have larger fasting and post-prandial gallbladder volumes, suggesting that both stasis and incomplete emptying contribute to sludge and stone formation [7]. Other factors contributing to gallstone formation include Gilbert's Syndrome and G6PD deficiency. Unconjugated bilirubin levels are substantially higher in SCD patients with the UDP-glucuronyltransferase isoform A1 (UGT1A1) genetic defect of Gilbert’s Syndrome (GS) [8]. SCD patients with concomitant GS are at an increased risk for biliary sludging with secondary gallstone formation [9]. G6PD deficiency, an X-linked disorder, leads to red cell destruction when older, enzyme-deficient cells are exposed to certain drugs, infections, or metabolic abnormalities. In the last thirty years, the relationship between G6PD and SCD has been the topic of a number of reports. Different studies have suggested beneficial, deleterious, or no effect on SCD course. A 1975 Nigerian study revealed no appreciable influence of G6PD deficiency on the expression of SCD [10]. These results were validated by a Jamaican study that found “no demonstrable correlation between clinical severity…and abnormal G6PD status” [11]. Finally, the Cooperative Study of Sickle Cell Disease (CSSCD) most recently found no evidence that G6PD enhanced hemolysis severity [12].

METHODS

Study site and subjects
A retrospective case-control study was performed on all pediatric sickle cell patients (type SS or S Sthal) who underwent cholecystectomy at the Children’s Hospital of Philadelphia from January 1993 to December 2008. Controls were age-matched patients who had not undergone cholecystectomy. Patient records were assessed for disease type, systemic symptoms related to gallstones, date of surgical procedure, post-operative complications, additional treatments, and sickle cell course, focusing on vaso-occlusive episodes (VOE), priapism, stroke or conditional/abnormal transcranial doppler (TCD), and development of pulmonary hypertension as measured by tricuspid regurgitation (TR) jet velocity. Clinical data, including age, sex, routine laboratory studies, imaging studies, and disease management, was also reviewed. The Institutional Review Board of the University of Pennsylvania, Children’s Hospital of Philadelphia, approved the study and the protocol for subject recruitment.

Statistical Methods
Two sample t tests or Wilcoxon rank sum tests were performed to compare numerical variables, including age, TCD, VOE admits, white blood cell count, hemoglobin, reticulocyte count, and LDH between groups (case versus control). Chi square tests were used to determine if there was a significant association between groups and categorical variables, including gender, priapism, stroke, pulmonary HTN, asthma, and chronic hypoxia. In addition, logistic regression was conducted to determine the effect of group on predicting stroke while controlling for hemoglobin level. General estimating equation method was used to determine the effect of group on predicting TCD and VOE treatment. All statistical analyses were performed with SAS (version 9.1; Cary, NC).

RESULTS (Table 1)
138 SS and 4 S Sthal patients underwent cholecystectomy during the study period. Of those, 113 records were available for review. For control subjects, 121 age-matched patients were available for review. There was no significant gender difference between the 2 groups with 57 males (50%) and 56 females (50%) in the case group and 63 males (52%) and 58 females (48%) in the control group. The average age at time of cholecystectomy was approximately 13 years. Approximately 75% of cases (n=85) were symptomatic prior to undergoing cholecystectomy. 12% (n=14) had frank cholecystitis. Interestingly, 18% (n=20) of cases were on a chronic transfusion protocol at the time of cholecystectomy for SCD-related complications, whereas only 7.5% (n=9) of controls were on a chronic transfusion protocol. 50% of cases were on chronic transfusions for secondary stroke prevention, 15% for primary stroke prevention (i.e. abnormal TCD), 15% for recurrent ACS, and 5% for recurrent splenic sequestration. The remainder (n=3) were on a chronic transfusion protocol for unknown reasons.

Non-SCD Comorbidities
2% of cases (n=2) and 1% of controls (n=1) carry a diagnosis of G6PD deficiency (p=0.61). There was a difference in the prevalence of GS, with a confirmatory abnormality in 5% of cases (n=2) and 67% of controls (n=2); however, GS testing was not performed in 81% of the total subjects (only 43/113 cases and 3/121 controls had GS testing). Asthma was a comorbid diagnosis in 14% of cases (n=16) and 16% of controls (n=20) (p=0.67).

Pulmonary Hypertension
Statistical differences in TR jet velocity and overt pulmonary hypertension were unable to be determined given that the majority of both cases and controls did not have an echocardiogram performed. Of the 113 cases, 43% (n=49) had an echocardiogram – 71% (n=35) of those did not have evidence of an elevated TR jet velocity and 29% (n=14) had an elevated TR jet velocity (mean 2.7 m/s, range 2.5-3.1 m/s). 33% of controls (n=40) had an
echocardiogram – of those, 77% (n=31) had a normal echocardiogram and 23% (n=9) had an elevated TR jet velocity (mean 2.8 m/s, range 2.5-3.2 m/s).

Priapism
There was no significant association between the number of subjects with priapism and group (p=0.47).

Stroke
Cases were 5.3 times more likely to have a stroke (p=0.02) while controlling for hemoglobin level. Cases were 2.2 times more likely to have a conditional or abnormal TCD (p=0.01). Of the 12 cases who had a history of stroke, 75% (n=9) occurred before cholecystectomy and were not associated with the cholecystectomy. One patient developed a severe headache with nausea/vomiting immediately following cholecystectomy and imaging of the brain showed an acute stroke with subarachnoid hemorrhage. The remaining cases (n=2) had their stroke event 2-3 years after cholecystectomy.

VOE
Mean VOE admissions for cases and controls were 5.1 and 2.5, respectively (p=0.002). Cases were 1.7 times more likely to receive IV pain medication or patient-controlled analgesia (PCA) for pain control (p=0.03).

Laboratory Studies
With regard to laboratory values, in the 3-6 year age group (n=10), the mean WBC count was significantly different: 15,000/microliter in the cases versus 10,900/microliter in the controls (p=0.004). For all age groups, mean WBC count was higher in the cases (p=0.05) but mean reticulocyte count was higher in the controls (p=0.004). Other pertinent laboratory values, including mean hemoglobin and mean LDH, were not significantly different between the cases and controls.

DISCUSSION
Chronic hemolysis, with release of hemoglobin from red blood cells, leads to increased bilirubin levels and subsequent gallstones. However, there are no published articles focusing on gallbladder pathology secondary to chronic hemolysis in pediatric SCD. This is the first paper to link another clinical marker of hemolysis, cholelithiasis, with SCD cerebral vasculopathy. In our patient cohort, children undergoing cholecystectomy for symptomatic gallbladder disease had an increased frequency of stroke and greater likelihood of having a conditional or abnormal TCD. Stroke occurs in 11% of SCD patients by 18 years of age and is the most severe complication affecting children with SCD. Bernaudin and colleagues published data from their SCD cohort on independent risk factors for abnormal TCD in pediatric patients and found LDH to be an independent predictor for high TCD velocities, suggesting that cerebral vasculopathy may be linked to hemolysis and NO bioavailability [13]. The SCD group at King’s College Hospital found that high LDH levels in children with SCD-SS correlated with abnormal TCD measurements [14]. LDH levels strongly correlate with level of hemolysis and, given our results, cholelithiasis may be another useful link to hemolytic rate. Interestingly, in our cohort, LDH levels were not significantly different between cases and controls. Several groups have looked at comorbid factors influencing rate of cholelithiasis in the SCD population and it has been established that UGT1A1 promoter polymorphisms do influence bilirubin levels and the development of gallbladder disease in the pediatric SCD population [9,15]. GS testing is not yet standard of care at our institution so our data was limited, but it will be beneficial to obtain that information for future clinical care.

Children undergoing cholecystectomy for symptomatic gallbladder disease had a higher number of VOE admissions and were more likely to require increased treatment. Hemolysis produces a young red cell population that is capable of adhesion and may result in polymer formation in cells leading to microvascular occlusion. A younger red cell population also has the capability of rapidly dehydrating cells under acid conditions, thus promoting hemolysis and adhesion [16]. SCD patients with symptomatic cholelithiasis may have increased VOE because of a baseline red cell population that promotes cellular adhesion and vascular occlusion.

Cases in the youngest age group (3-6 years) had increased SCD morbidities as compared to their age-matched controls, including increased episodes of ACS, splenic sequestration, and chronic hypoxia. This is a very interesting finding and warrants additional investigation.

Our study was limited in that we had restricted ability to capture patients with asymptomatic gallstones as routine abdominal ultrasonography is not standard of care at our institution. We may have missed a rare case of symptomatic gallbladder disease not undergoing cholecystectomy. Additionally, in a retrospective study, missing data, such as echocardiogram results, can hinder overall analysis.

In summary, we found that early gallbladder disease may be a marker of increased clinical severity. Gallbladder pathology in SCD patients deserves further study, particularly in decision-making regarding elective cholecystectomy for asymptomatic cholelithiasis as well as the early initiation of treatments to reduce the level of hemolysis in this patient population.
REFERENCES


ACKNOWLEDGEMENTS: The authors thank Henrietta Enninful-Eghan and Tannoa Jackson for their assistance in data collection and chart review. This work was supported by a hematology training grant from the National Institutes of Health (ST32HL007150-32).
Table I. Clinical and Laboratory Data

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=113)</th>
<th>Controls (n=121)</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
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<td>0.80</td>
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<tr>
<td>Male</td>
<td>50% (n=57)</td>
<td>52% (n=63)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>50% (n=56)</td>
<td>48% (n=58)</td>
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</tr>
<tr>
<td><strong>Mean Age (yrs) (SD)</strong></td>
<td>12.7 (±4.5)</td>
<td>13 (±4.4)</td>
<td>0.55</td>
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<tr>
<td><strong>Stroke</strong></td>
<td>11% (n=12)</td>
<td>4% (n=4)</td>
<td>0.06</td>
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<tr>
<td><strong>Highest TCD</strong></td>
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</tr>
<tr>
<td>Normal</td>
<td>73% (n=82)</td>
<td>81% (n=97)</td>
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</tr>
<tr>
<td>Conditional</td>
<td>16% (n=18)</td>
<td>15% (n=18)</td>
<td>0.82</td>
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<tr>
<td>Abnormal</td>
<td>12% (n=13)</td>
<td>5% (n=6)</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Priapism</strong></td>
<td>16% (n=9)</td>
<td>11% (n=7)</td>
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<td><strong>Pulmonary HTN</strong></td>
<td>29% (n=14)</td>
<td>23% (n=9)</td>
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<td><strong>Asthma</strong></td>
<td>14% (n=16)</td>
<td>16% (n=20)</td>
<td>0.67</td>
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<tr>
<td><strong>Chronic Hypoxia</strong></td>
<td>10% (n=11)</td>
<td>11% (n=14)</td>
<td>0.69</td>
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</tbody>
</table>

**Mean (±SD)**

<table>
<thead>
<tr>
<th></th>
<th>Mean (±SD)</th>
<th>Mean (±SD)</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td><strong>Mean WBC Count (SD)</strong></td>
<td>12,300/microliter (±3400)</td>
<td>11,500/microliter (±2900)</td>
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<td><strong>Mean Hgb (SD)</strong></td>
<td>8.4 g/dL (±1.0)</td>
<td>8.6 g/dL (±1.2)</td>
<td>0.24</td>
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<tr>
<td><strong>Mean Retic Count (SD)</strong></td>
<td>11.8% (±4.3)</td>
<td>13.5% (±4.7)</td>
<td>0.004</td>
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<tr>
<td><strong>Mean LDH (SD)</strong></td>
<td>1455 U/L (±485)</td>
<td>1497 U/L (±504)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

†Reflects highest severity category per subject, ‡Missing data (only 49 cases and 40 controls had an echocardiogram)
SD = Standard Deviation; TCD = Transcranial Doppler; HTN = Hypertension; WBC = White Blood Cell;
Hgb = Hemoglobin; LDH = Lactate Dehydrogenase
JSCDH-D-15-00097

A validated measure of adherence to antibiotic prophylaxis in children with sickle cell disease

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Key words: sickle cell disease, penicillin prophylaxis, antibiotic prophylaxis, prophylaxis, adherence

Abstract (125 words)

Background: Antibiotic prophylaxis is a mainstay in SCD management, however, adherence is estimated at only 66%. This study aimed to develop and validate a Sickle Cell Antibiotic Adherence Level Evaluation (SCAALE) to promote systematic and detailed adherence evaluation.

Methods: A 28-item questionnaire was created, covering 7 adherence areas. General Adherence Ratings from the parent and one healthcare provider and medication possession ratios were obtained as validation measures.

Results: Internal consistency was very good to excellent for the total SCAALE (α=0.89) and 4 of the 7 subscales. Correlations between SCAALE scores and validation measures were strong for the total SCAALE and 5 of the 7 subscales.

Conclusions: The SCAALE provides a detailed, quantitative, multidimensional and global measurement of adherence and can promote clinical care and research.

Background

Children with sickle cell disease (SCD) have compromised splenic function that increases the rate of bacteremia with the ensuing risk of rapid progression to sepsis, septic shock, and death.[1, 2] Penicillin prophylaxis has become a mainstay in the management of children with SCD to prevent complications relating to infection.[3] With the implementation of prophylactic antibiotic prescribing, the risk of bacteremia in febrile children with SCD has decreased from 3-5% as documented from 1975-2002.[4-10] to <1%.[2, 11]

Yet in spite of penicillin’s effectiveness, adherence to the twice-daily treatment regimen is of enduring concern.[12-22] Low adherence rates illustrate the need for an ongoing and systematic method to measure and understand adherence in this community with the goal of improving treatment outcomes. Even a low incidence rate can affect a numerically large group of children when a disorder is as prevalent as SCD,[23, 24] and rates of bacteremia as a result of nonadherence may be higher in certain high-risk subpopulations affected by SCD. Therefore, measurement and improvement of adherence offers the potential for documenting, understanding, and improving outcomes in high-risk subpopulations and further reducing the number of bacteremia cases associated with SCD.

Several methods have been used to measure adherence in SCD. These include medical record reviews,[20] urinalysis,[13, 14, 17, 20] self/parent-report via questionnaires, interviews, or visual analogue scales,[14, 18, 20, 25] parent/proxy Morisky scores,[25] medical provider reports,[25], clinic visit attendance,[25] medication event monitoring system (MEMS) pill bottles,[15] and medication possession ratios (MPRs) using pharmacy claims,[12, 16, 22, 26] or dispensation data.[19, 21, 25] Most of these measures have been validated in other clinical populations, yet the information we can glean from
them is limited. As Beverung et al, who utilized an MPR,[12] state, "...we cannot explain why adherence is low..." The MPR, for instance, produces an adherence rating, but does not provide information on (potentially modifiable) variables underlying nonadherence. Additionally, MEMS and MPRs do not provide information about whether the medicine was actually given to the child or under what conditions. Furthermore, medical record reviews, urinalysis, MEMS, and MPRs are costly and time-intensive to obtain, reducing their feasibility for daily clinical use at a population level. The lack of an economically and clinically feasible instrument to measure penicillin prophylaxis adherence in SCD has resulted in almost no systematic, population-wide documentation or monitoring of adherence in daily clinical practice, despite the well-established value of penicillin prophylaxis for preventing infection in this at-risk population.

This critical gap in our understanding of adherence to penicillin prophylaxis (and application of adherence knowledge to the daily clinical setting) exposes a need in SCD clinical management and research for a more feasible, clinically relevant, multidimensional measure of adherence, i.e., one that has more layers of information than just a single, global score. In light of the need for potential benefit of a new multidimensional measure of adherence that is valid, low-cost, and provides real-time information based on the perspective of the individual most responsible for adherence (the caregiver), we developed and validated the Sickle Cell Antibiotic Adherence Level Evaluation (SCAALE). This study aimed to describe the psychometric properties and validity of the SCAALE and to demonstrate its potential utility as a clinical and research instrument. Developed using a conceptual framework of adherence first utilized in hemophilia, [27, 28] the SCAALE is a brief parent/guardian-report questionnaire designed to evaluate specific areas of adherence, identified as subscales, as well as global adherence to antibiotic prophylaxis.

**Methods**

**Recruitment and procedures**

The study protocol was approved by the St. Vincent Hospital Institutional Review Board (IRB). Patients were recruited by Sickle SAFE (Screening, Assessment, Follow-up, and Education) Program coordinators during home visits or by telephone. All participants provided informed consent; parents/guardians consented for minor children. Participants also signed a release of information, granting permission to contact the patient’s pharmacy and obtain dispensation records for the year preceding questionnaire completion. The parent/guardian was then given the SCAALE with a demographic cover sheet and allowed as much time as necessary to complete the survey.

**Participants**

Participants were recruited from the population of patients enrolled in the Sickle SAFE Program, the Indiana State Department of Health-supported hemoglobinopathy newborn screening (NBS) follow-up program. This program ensures timely notification of affected patients, educates families about the confirmed diagnosis and management of the disease, and links families to a hematologist. All infants in Indiana identified by the NBS laboratory as having a hemoglobinopathy are enrolled in the Sickle SAFE Program. From that population, we recruited only patients diagnosed with hemoglobin SS disease (Hgb SS), S beta thalassemia* (Hb S/β^0^ Th), S beta thalassemia^0^ (Hb S/β^0^ Th), or hemoglobin SC disease (Hgb SC) who had been prescribed twice daily antibiotic prophylaxis for at least three months (the recall period on the questionnaire). The study was limited to English-speaking patients under 6 years of age.

**Measure**

The SCAALE is a 28-question survey divided into seven (four-question) subscales: Time, Dose, Pharmacy, Plan, Remember, Communicate, and Environment. An eighth (five-question) subscale, Other Caretakers, was piloted; it remains under further review and is excluded from this report. Questions and subscales were rationally developed and revised in a five-step process to optimize content validity: (1) initial question development by hemoglobinopathy care specialists; (2) questions review by a parent/guardian focus group; (3) question revision and addition of two subscales (‘Pharmacy’ and ‘Other Caretakers’) based on focus group feedback; (4) SCAALE administration to a 34-patients pilot sample for preliminary reliability and validity analysis; and (5) based on pilot sample results, slight modifications to some questions and to descriptive anchors for the scoring scale to improve sensitivity. Questions were written to reflect the caretaker’s actions and experiences managing a twice-daily antibiotic schedule.

SCAALE response options are five-point Likert scales (‘Always,’ ‘Almost Always,’ ‘Often,’ ‘Sometimes,’ ‘Rarely or Never’). An answer of ‘Always’ reflects the ‘best’ possible adherence for some questions and the ‘worst’ possible adherence for others. Questions are scored so that responses indicating ‘worst’ adherence receive one point while responses indicating ‘best’ adherence receive five points. SCAALE subscale and total scores are the averages of the questions comprising them and range from 1 (least adherent) to 5 (most adherent). The survey asks the respondent to report adherence for the past three months.

**Validation measures of adherence**

Three adherence measures were used to demonstrate SCAALE construct validity: General Adherence Rating (GAR) by parent, GAR by healthcare provider, and medication
possession ratio (see descriptions below). Because no single adherence measure can provide a perfect characterization of adherence (short of a behavior coder who directly observes the child constantly), an adherence measure’s validity is best captured by demonstrating significant relations between the adherence measure and several other estimates of adherence.

**General adherence rating**
The demographic cover sheet included a GAR scale on which respondents rated their global adherence level using a scale of 1 (‘rarely or never’ follows the doctor’s instructions for antibiotic use) to 10 (‘always’ follows the doctor’s instructions for antibiotic use). The parent completing the SCAALE and one healthcare provider per subject provided a GAR.

The healthcare provider completing the GAR was the individual who most closely follows the patient’s prophylactic antibiotic treatment for SCD. Provider responses were based on a global impression of the family’s adherence. Similar provider rated global impression scales are widely used in medicine, including visual analogue scales[29] and global impression ratings.[30]

The GAR has been validated in previous adherence research.[27, 28]

**Medication possession ratio (MPR)**
The medication possession ration (MPR) is a widely used adherence measure and has been used in several studies evaluating antibiotic adherence in SCD.[12, 16, 19, 21, 22] Advantages of the MPR are that it does not rely on self-report and the data can be obtained from known sources. However, limitations exist. Refilling a prescription is not the same as ingesting it, and MPR is best calculated in a closed pharmacy system,[31] which was not available for this study.

An MPR for the 3 months preceding SCAALE completion was calculated using pharmacy dispensation records. The MPR was the ratio of the number of days during which the patient had antibiotics as indicated by the number of doses dispensed (numerator) to the number of days in the study period (90 days, denominator). An MPR of 1.0 indicates 100% adherence in terms of prescription refills relative to medication prescribed, while lower MPRs indicate that less medication was dispensed than was prescribed (lower adherence). In some cases, MPRs above 1.0 were observed. To reflect that MPRs >1.0 did not necessarily indicate greater adherence than MPRs=1.0, MPR values >1.0 were re-coded as 1.0.

**Statistical analysis**
The SCAALE development process followed standardized methodology and a protocol utilizing commonly accepted statistics for validation studies.[32, 33] The statistics reported are outlined below.

**Descriptive Statistics for SCAALE Item, Subscale, and Total Scores**
Descriptive statistics and distributions (mean, range, SD) of SCAALE total and all subscale scores are provided. Because most subjects report adherence to a medical regimen, it is not uncommon for scores to cluster at the upper end of the distribution.[27, 28] A very significant clustering of scores at the upper end of the distribution represents a ceiling effect. For SCAALE scores, ceiling effects were defined as either 90% of the answers on any question being the highest possible option (i.e., ‘5’) or SD<0.5 and mean>4.8, which would indicate restricted variance.

**Subscale Intercorrelations**
Subscale intercorrelations were calculated to investigate relations among the different areas of adherence measured by SCAALE subscales. Strong subscale intercorrelations demonstrate convergent validity, by showing the subscales measure the same construct. Intercorrelations are reported as Pearson product-moment correlation coefficients (r), which range from -1.0 to +1.0; values closer to 0 reflect weaker relationships.

**Internal consistency reliability**
Internal consistency reliability (ICR) is a measure of whether a group of questions evaluate the same defined concept. This was assessed for the total SCAALE and all subscales and is reported as Cronbach’s alpha (α). This statistic ranges from 0.0 to 1.0; the closer to 1.0, the stronger the ICR. Given the short subscale length (4 questions), making higher α more difficult to achieve, α≥0.8 was considered to reflect
excellent ICR; [$\alpha = 0.7$ to 0.79], very good; [$\alpha = 0.6$ to 0.69], good; and [$\alpha = 0.5$ to 0.59], minimally acceptable.

**Correlations with validity measures**

A valid SCAALE must accurately reflect adherence, shown by shared variance with other estimates of adherence. One test of this is a correlation between the scale score and the validity measure (either GAR or MPR). Correlations with validity measures are reported as Pearson product moment correlation coefficients ($r$) and associated $p$ values.

**Results**

**Demographics**

88 families were recruited; 21 declined, primarily due to lack of interest in the study or a desire for privacy. An additional 7, who were consented by telephone, did not return mailed questionnaires. Of the 60 remaining, two participants were excluded due to age >6 years. This resulted in a sample size of 58. See Table 1 for sample demographic and medical information.

**Question- and Subscale-level descriptive data and intercorrelations**

At a question level, significant ceiling effects were found for 9/28 questions (3 questions each from the ‘Dose’, ‘Pharmacy’, and ‘Environment’ subscales). All score distributions were skewed negatively, i.e., most data were at the high end of the distribution.

Mean total SCAALE score was 4.7, with a range of 3.14 to 5.00 (Table 2). Subscale mean scores ranged from 4.4 (‘Plan’) to 4.9 (‘Dose’ and ‘Environment’). The ‘Time’, ‘Plan’, and ‘Environment’ subscales had the highest median intercorrelations with the other 6 subscales (0.53, 0.43, and 0.40, respectively), whereas the ‘Pharmacy’ (0.33) and ‘Communicate’ (0.14) subscales had the lowest median intercorrelations. The SCAALE total score was significantly correlated with all subscales (median correlation=0.64, range=0.50 [Communicate] to 0.87 [Plan]; a Table of all intercorrelations is available from the authors).

**Internal consistency reliability (ICR)**

ICR for the total scale was excellent at $\alpha=0.89$ (Table 2). Subscale ICRs were variable, ranging from excellent 0.86 (‘Time’), 0.83 (‘Communicate’), and 0.82 (‘Plan’); to very good 0.77 (‘Remember’); to poor 0.22 (‘Environment’), 0.24 (‘Pharmacy’), and 0.32 (‘Dose’). Notably, subscales with poor ICR are also those containing the most significant ceiling effects.

**Validity adherence measures**

Consistent with SCAALE question and subscale ratings, GAR measures were significantly negatively skewed (i.e., toward the high end of the distribution). Fifty-seven (98%) parents provided GARs, with 89% rating their adherence 9 or 10 (mean 9.5, SD 1.05). Healthcare provider GARs were given for 33 (56%) participants, with 61% receiving a score of 9 or 10 (mean 8.8, SD 1.25). Healthcare providers included primary care providers (PCPs) (n=22, 23 subjects), hematologists (n=4, 6 subjects), and PCP with a focus on hematology (n=1, 4 subjects). Parent GAR correlated significantly with Provider GAR (0.48, $p<0.01$).

MPRs were calculated for the 37 (64%) participants for whom pharmacy dispensation data was available. MPR ranged from 0.11 to 1.00 (mean 0.65, SD 0.30). Only 38% of the sample had MPRs greater than 0.80, while 19% of the sample had MPRs of 0.33 or less. MPR correlated significantly with provider GAR ($r=0.57$, $p<0.02$) but not with parent GAR ($r=0.24$, $p<0.15$).

The SCAALE total score correlated significantly with parent GAR ($r=0.69$, $p<0.01$), provider GAR ($r=0.44$, $p<0.05$), and MPR ($r=0.46$, $p<0.01$). The majority of SCAALE subscales also correlated significantly with two or more of the validity measures, and the ‘Plan’ subscale correlated significantly with all three validity measures. All SCAALE subscales with the exception of ‘Pharmacy’ and ‘Communicate’ correlated significantly with Parent GAR. Provider GARs were significantly correlated with the ‘Dose’ and ‘Plan’ subscales, and correlations between Provider GAR and the ‘Time’ and ‘Communicate’ subscales were high ($r<0.06$). ‘Time’, ‘Plan’, and ‘Environment’ were significantly correlated with MPR (Table 3).

**Discussion**

Daily oral administration of prophylactic penicillin has significantly reduced mortality associated with bacterial infections in children with SCD.[2, 11, 34] This treatment is recommended by the National Heart, Lung and Blood Institute[35] as a standard of care for children with Hb SS and Hb S/β Th under 5 years of age and in older children who have had a previous severe pneumococcal infection or have functional/surgical asplenia. It was also identified in 2011 by a Sickle Cell Disease Expert Panel as a quality of care indicator rated 9 out of a possible 10 for importance.[36] However, in spite of these endorsements, the effectiveness of antibiotic prophylaxis for young children with SCD may be limited by nonadherence to the treatment recommendations of twice daily administration. There does not currently exist a widely accepted, validated, clinically useful means specifically designed to measure the multiple dimensions of prophylactic antibiotic adherence in SCD. This study aimed to address this gap by developing and validating a standard measure of global and specific dimensions of prophylactic antibiotic
adherence in SCD; the Sickle Cell Antibiotic Adherence Level Evaluation (SCAALE).

In addition to providing a global view of adherence based on a total score, the SCAALE contains seven subscales that yield more specific and detailed descriptions of different aspects of adherence. Based on prior empirical research[27, 28] and focus group data, adherence is not a simple unitary construct but rather consists of, and is driven by, multiple related factors such as timing, dosing, planning, and access to medical care. Therefore, measuring dimensions of adherence in addition to a total score is important for understanding the underlying contributors and components of nonadherence, providing a first step toward targeted interventions for at-risk families.

The complete 28-item SCAALE total score has both the strongest ICR and the strongest validity correlations with global measures of adherence as rated by parents, providers, and MPR. This is not surprising as the SCAALE total score captures all dimensions of adherence in a single measure, whereas subscales focus on specific areas of adherence that may be more important in some families and less important in others. Additionally, the SCAALE total score has a broader distribution and larger variance than the subscales because it consists of more items across multiple adherence areas. Finally, longer scales generally have larger ICR values than shorter scales because Cronbach’s α is partially dependent on the length of the scale.[32]

Reliability and validity of the subscales were variable, with some subscales showing strong ICR and validity (‘Time’, ‘Plan’, ‘Remember’, and ‘Communicate’), and others showing questionable ICR but significant validity correlations (‘Dose’ and ‘Environment’). The ‘Pharmacy’ subscale, on the other hand, had poor ICR and low validity correlations, suggesting a need for additional research. The ‘Pharmacy’ subscale is nevertheless recommended for inclusion in the SCAALE because of its content validity based on unanimous recommendation by focus group participants and experts in SCD pediatric clinical practice.

Parents and providers rated the study sample as highly adherent, as measured by GARs and the SCAALE. Although such high adherence ratings are desirable from a clinical perspective, they limit the power of psychometric analyses by introducing restricted range and ceiling effects into the analysis. As noted under Internal Consistency Reliability, subscales with near-ceiling effects (‘Dose’, ‘Pharmacy’, ‘Environment’) had low ICR, likely reflecting insufficient variability in the sample data as opposed to poor quality of the subscales – a larger sample and further validation analysis are needed to address this question. Subscales with greater variability (‘Time’, ‘Plan’, ‘Remember’, ‘Communicate’) had good-to-excellent ICR.

Of the subscales, ‘Plan’ and ‘Time’ were most consistently and significantly related to the three validity measures. This suggests that across the entire sample, behaviors related to planning to have antibiotics available and administering them at the proper time are especially important for global adherence, and therefore should be core components of adherence measurement and intervention in this population.

Our overall findings lend themselves to several implications and recommendations. The total scale score was the most reliable, valid, and best index of global adherence, reflecting its integration of multiple dimensions of adherence. It showed very strong ICR and correlations of 0.44 or higher with Parent GAR, Provider GAR, and MPR. GAR scores provided by the parent did not correlate significantly with MPR (r=0.24, p=0.14), while SCAALE total scores based on parent report did correlate significantly with MPR (r=0.46, p=0.004). This finding indicates that measuring global adherence based on a sum of the specific domains evaluated by the SCAALE is superior to obtaining a single global estimate provided by the parent. Such a finding also demonstrates that the core domains of the SCAALE reflect critical adherence components related to MPRs.

Our approach to SCAALE validation emphasized its relationships with multiple other methods of estimating adherence, each of which has advantages and limitations. None of the validating measures used in this study is without limitations, nor do we claim that the SCAALE is a perfect method for determining adherence. Rather, parent-report of adherence has specific advantages and contributions to estimating adherence that cannot be obtained with other methods such as MPR or GAR. Furthermore, if adherence interventions are to target parents, it is critical to understand the components and barriers to adherence based on their report. As a result, the SCAALE has a significant and important role as a parent-report measure of adherence to penicillin prophylaxis. We took a multisource (parent- versus provider-report), multi-method (questionnaire and prescription record) approach to obtaining other validity measures of adherence for the SCAALE, demonstrating significant relationships among adherence estimates based on different methods and sources.

Although healthcare provider ratings of adherence have limitations, they are correlated (albeit modestly) with methods of estimating adherence. For instance, Logan et al (2003) found significant relationships between provider-reported estimates of adherence and patient adherence to different domains measured using the Illness Management Survey.[37] Zeller et al (2008) found a significant correlation between the physicians’ predictions of adherence and MEMS measures of adherence.[38] Because healthcare providers see a wide range of patients, they may be able to detect extremes in adherence at above chance levels, and their perspective on adherence is
valuable since it is likely to influence their medical decision-making and interactions with patients. [39]

Importantly, the high level of adherence in our sample may in part reflect the degree of resources devoted to this patient group, which is actively monitored, managed, and supported by a structured newborn screening (NBS) follow-up program. Upon receiving an abnormal hemoglobin result from the NBS laboratory, a Sickle SAFE Program Coordinator contacts parents by telephone to discuss the diagnosis and schedule a home visit. The coordinator also contacts the PCP to provide education on the importance of antibiotic prophylaxis and ensure the first prescription for antibiotic prophylaxis is written. At the first home visit, when the patient is approximately 3 to 6 weeks of age, the coordinator delivers the first 3-months’ supply of penicillin, provided free of charge. During the same home visit, the coordinator provides education and training on antibiotic reconstitution and dose administration. Sickle SAFE participants receive regular communications from the coordinator and a direct line remains open for participants to contact Program staff, which includes the coordinator and a pediatric hematologist. Moreover, if a participant loses insurance coverage, the Sickle SAFE Program provides penicillin for the uninsured period at no cost to ensure continuity of care. Research has shown that such patient-centered interactions promote adherence and lead to improved health outcomes. [40] It is quite possible that lower levels of adherence would be reported in samples who do not receive this level of support.

Some methodological considerations should be taken into account when interpreting results of this study. First, although the sample size of 58 was sufficient for psychometric analysis, a larger sample would likely yield greater variability among scores and may result in stronger reliability and validity statistics. It is possible that some of the weaker reliability statistics were a result of insufficient variability.

A second consideration is the skewed distribution of SCAALE scores. We believe that this reflects the tendency of this particular sample to be adherent due to frequent, patient-centered interactions with the Sickle SAFE Program Coordinator. While this may be a positive reflection on that program, a less adherent sample could produce stronger reliability and validity correlations by providing a larger range of scores and wider distribution within the range. Similarly, of those patients enrolled in the Sickle SAFE Program, it is possible that only the most adherent chose to participate, introducing selection bias.

A third methodological consideration is the quality of the pharmacy dispensation data available for calculating MPRs. In spite of multiple telephone follow-ups to patients’ pharmacies, dispensation records were available for only 37 (64%) study participants. Of the data obtained, we were unable to differentiate instances of missing data (due to pharmacy error or the failing to provide a comprehensive list of pharmacies) from when patients were actually missing dispensations (due to nonadherence). To be as thorough as possible, when data were missing, a second attempt was made to gather the data by going back to the patient and pharmacy to check for errors in record provision or pharmacy telephone numbers.

A fourth consideration in the interpretation of the SCAALE is the rating method, which is based on parent-report. Parent reports are susceptible to bias and error ranging from social desirability to denial to poor self-awareness and self-monitoring. Furthermore, significant correlations between SCAALE scores and Parent GARs may be influenced by method bias because both were completed by the same rater. For this reason, we obtained adherence validity scores from three critical perspectives: parent, provider, and pharmacy dispensation data. Importantly, SCAALE total scores correlated with all three types of validity measures, demonstrating that method bias from parent-report does not account for the validity results.

While the SCAALE demonstrates strong psychometric properties and fills a critical unmet need, additional research is needed to address some shortcomings. Additional planned scale development initiatives are a test-retest stability investigation and use of the scale with a large, diverse set of treatment centers with varying NBS follow-up programs.

The SCAALE provides the first detailed, quantitative, dimensional and global measurement of adherence to antibiotic prophylaxis in SCD. Evidence from this study supports the reliability and validity of the overall 28-question scale and of most subscales. Development of this scale represents an important contribution to pediatric SCD with clear applicability to clinical management, research programs, and state-funded NBS initiatives.

Acknowledgement

The authors wish to acknowledge the Indiana State Department of Health and the Indiana Hemophilia and Thrombosis Center, Inc. for their ongoing funding and support of the Sickle SAFE Program.
References


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Sickle Cell Disease in Pregnancy in a Nigerian Tertiary Health Center: Our Challenges and Strengths

Authors: Collins Ogbonna Nwabuko, MD, FMCPath, Dorathy Adaunwa Okoh, MD, FWACP, Caroline Lyalla, MBBS, FMCPath

Affiliation: Federal Medical Center/ Abia state University, Umuahia, Abia NIGERIA

Table 1: Genotype (Hemoglobin Variants) of Pregnant Women @ Booking

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency (n)</th>
<th>Percent (%)</th>
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<tbody>
<tr>
<td>AA</td>
<td>28,815</td>
<td>80.09</td>
</tr>
<tr>
<td>AS</td>
<td>7,109</td>
<td>19.77</td>
</tr>
<tr>
<td>SS</td>
<td>52</td>
<td>0.14</td>
</tr>
<tr>
<td>Total</td>
<td>35,976</td>
<td>100.00</td>
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Table 2: Levels of Education of Pregnant Women @ Booking

<table>
<thead>
<tr>
<th>Level of Education</th>
<th>Frequency (n)</th>
<th>Percent (%)</th>
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<tbody>
<tr>
<td>Primary (1-6)</td>
<td>71</td>
<td>0.2</td>
</tr>
<tr>
<td>Junior Secondary School (JSS 1-3)</td>
<td>811</td>
<td>2.4</td>
</tr>
<tr>
<td>Senior Secondary School (SSS1-3)</td>
<td>13,417</td>
<td>39.5</td>
</tr>
<tr>
<td>Tertiary (University/Polytechnic)</td>
<td>19,447</td>
<td>57.3</td>
</tr>
<tr>
<td>Post-Graduate (Masters/Ph.D.)</td>
<td>190</td>
<td>0.6</td>
</tr>
<tr>
<td>Total</td>
<td>33,939</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Note: Only the levels of Education of 33,939 registered pregnant women were documented

Table 3: Pattern of Hemoglobin Concentrations of Pregnant Women @ Booking

<table>
<thead>
<tr>
<th>Hb Concentration (g/dl)</th>
<th>Frequency (n)</th>
<th>Percent (%)</th>
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</thead>
<tbody>
<tr>
<td>&lt;11.0</td>
<td>13,416</td>
<td>39.4</td>
</tr>
</tbody>
</table>
Note: <11.0 g/dl connotes Anemia in pregnancy \([^{15,16}]\). 34,020 of the registered women had their Hemoglobin concentration documented.

Table 4: Cross tabulation of Hemoglobin Concentrations with Genotypes in Pregnant women @ Booking

<table>
<thead>
<tr>
<th>Hb concentration (g/dl)</th>
<th>AA</th>
<th>AS</th>
<th>SS</th>
<th>Total (n)/(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;11.0</td>
<td>10,262 (77.1)</td>
<td>3014 (22.6)</td>
<td>42 (0.3)</td>
<td>13,318 (39.3)</td>
</tr>
<tr>
<td>&gt;11.0</td>
<td>16,506 (80.3)</td>
<td>4048 (19.7)</td>
<td>10 (0.0)</td>
<td>20,564 (60.7)</td>
</tr>
<tr>
<td>Total</td>
<td>29,768 (79.0)</td>
<td>7062 (20.8)</td>
<td>52 (0.2)</td>
<td>33,882 (100.0)</td>
</tr>
</tbody>
</table>

Chi-Square: 188.78, p-value=0.001

Note: 33,883 of the registered pregnant women had their recorded hemoglobin concentrations and genotypes (Hb variant) documented.

Sickle Cell Disease in Pregnancy in a Nigerian tertiary health center: Our Challenges and Strengths

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Abstract

Background: Sickle cell disease (SCD) in pregnancy is gaining increase in popularity worldwide today because of the obstetric complications it poses if the pregnancy progresses without adequate care and follow-up. This study aimed at determining the prevalence and challenges of SCD among pregnant women seen in ante-natal clinic in a South-southern Nigerian tertiary health center.

Materials and Method: This was a ten-year retrospective study of thirty five thousand, nine hundred and seventy six pregnant women seen at the antenatal clinic of Braithwaite Memorial Specialist Hospital (BMSH) (2003-2013). Biomedical data and hemoglobin (Hb) electrophoresis were...
obtained using hypothesis generation questionnaires and conventional hemoglobin electrophoretic machines respectively. Data analysis was obtained using SPSS version 16.

Result: A total of 35,976 pregnant women registered in the ante-natal clinic within the study period out of which 28,815 (80.09%) were Hb variant AA, 7,109 (19.77%) were Hb AS, and 52 were SS (Hb SS-homozygous which is prevalence of 1.4 per 1000 pregnant women). 39.4% of the women had Hb concentration level below 11 g/dl (anemia), while 80.7% was above 11 g/dl (P=0.001).

Conclusion: The prevalence of sickle cell disease in pregnancy is on the increase in this region. Awareness creation and national policies that will scale up the care of sickle cell disease in pregnancy should be topmost priorities in improving their life expectancies in Nigeria.

**Keywords:** Sickle cell disease, pregnancy, Hemoglobin variant.

**Introduction**

One of the greatest Public health problems of our time is due to sickle cell disease, a genetic disease of the globin chain of the red blood cells. It affects millions of people throughout the world and is commoner among those whose ancestors came from sub-Saharan Africa. In West and Central Africa where it is the commonest hemoglobinopathy, 25% of the people have sickle cell trait while 2-3% of all babies are born with a form of the disease. It is estimated that sickle cell occurs in 1 out of every 500 African child. In United States, an estimated 80,000-90,000 Americans of African extraction are affected by sickle cell disease, while about 3 million have sickle cell traits. In Nigeria, about 45,000-90,000 babies with sickle cell disease are born annually as against 1000 babies born in United States.

The sickle cell (SC) gene disorder occurs in high frequency in endemic malaria regions especially in the Plasmodium falciparum pressure belt low and middle-income countries. These areas account for three-quarter of an estimated 300,000 to 500,000 children born with SC disease worldwide every year. The high prevalence stems from an evolutionary link between the SC gene and resistance to malaria, a feature that also underpins the common inclusion of Sickle Cell screening in health research in malaria endemic zones where the gene may act as a risk factor.

Sickle cell disease is reported to be associated with a very high rate of childhood mortality. It contributes to 5% of under-5 death on the African continent. Other health problems include: decrease in median survival, chronic anemia, end-stage renal failure, acute- chest syndrome, vaso-occlusive-, hyper-hemolytic, aplastic- and sequestration- crises. All these complications pose significant threats to public health in the management of sickle cell disease, especially in economic constrained settings such as those found in sub-Saharan Africa.

Pregnancy in patients with sickle cell disease is associated with increased maternal and fetal morbidity and mortality. Maternal complications may include recurrent anemia, bone pain crises, recurrent malaria infection, late menarche, acute chest syndrome, spontaneous abortion, lobar pneumonia, HIV, pseudo toxemia, hemolytic crises, pre-eclampsia, retained placenta and maternal mortality just to mention a few. The fetal complications may include: low birth weight (LBW), Intra-uterine Fetal death (IUFD), Stillbirth, breech presentation, etcetera.

Although progress has been made in the management of sickle-cell disease in high-income countries over the past one decade, in most middle- and low-income countries where sickle cell disease is a major public health problem, its management has remained inadequate. There are no strong national health policies to control sickle cell disease in those countries and so the basic facilities to screen, diagnose or manage the patients (Prophylactic antibiotics and immunization for example) are usually absent. More often, diagnoses are first made when complications have set in. Without early diagnosis and treatment, pregnant mothers and children with SCD often die due to labor complications and chronic anemia.

This study is aimed at determining the prevalence of sickle cell disease among pregnant women in South-Southern Nigerian and the relationship of the hemoglobin variants of the pregnant women with their Packed cell volume at booking. The result of this study will be used to generate policy programs that will improve the quality of life of people living with SCD in sub-Saharan Africa.

**Material and Method**
Study Site and Design: This study was a retrospective study of SCD burden among antenatal patients who were seen in antenatal clinic in BMSH community in Port Harcourt, Rivers State Nigeria. Port Harcourt is an oil-rich cosmopolitan city located in the Niger Delta region of Nigeria. It has an international airport and a sea port. The presence of oil attracted so many oil companies such as Shell petroleum development company (SPDC), Mobil, Chevron, and Agip oil companies just to mention a few into the city.

Study period: This was a retrospective study dated from 2003-2013.

Study population: A total of 35,976 pregnant women at different stages of gestation were seen during the study period.

Ethical clearance for the study was obtained from the ethical committee of the Braithwaite Memorial Specialist Hospital (BMSH).

Data Collection and Analysis: Data was collected through Health Information medical records from the files of the patients at the ante-natal clinic BMSH. There was a hypothesis generation questionnaire which was source of information at the booking clinic for each patient. Hemoglobin variant was obtained through Hemoglobin electrophoresis of patients venous blood collected at first booking in the antenatal clinic. All data were entered and analyzed using EPI info statistical software version 6.02. Statistical analysis of mean and standard deviation were calculated. Student t-test was used to test the significance of differences between mean values. A probability (p) less than 0.05 were taken to indicate statistical significance.

Selection Criteria: Pregnant woman irrespective of the age.

Result

A total of thirty five thousand, nine hundred and seventy six (35,976) women who registered at the ante-natal clinic of BMSH community, Port Harcourt between 2003 to 2013 were studied, out of which 28,815 (80.09%) were Hb variant AA (Hb AA), 7,109 (19.77%) were AS (Hb AS), and 52 were SS (Hb SS-homozygous) (table 1). Out of 33,939 of them who had their levels of education documented, 74 (0.2%) had Primary school education, 811 (2.4%) had Junior Secondary School Education, 13,417 (39.5%) had Senior Secondary School Education, 19,447 (57.3%) had Tertiary level of education, while 190 (0.6%) had Post-graduate level of education (Table 2). The Mean gestational age at booking was 29.18 (±4.69) weeks. The minimum gestational age of booking was at 11 weeks while the maximum GA was at 79 weeks. 13,416 (39.4%) presented with hemoglobin concentration less than 11.0 g/dl while 20,604 (60.6%) presented with hemoglobin value greater than 11.0 g/dl (Table 3). Out of 52 Hb variant SS (Hb SS) pregnant mothers recorded, 42 (80.8%) registered at hemoglobin concentration less than 11.0 g/dl while 10 (19.2%) were above 11.0 g/dl (p-value=0.001) (Table 4).

Discussion

This study showed 0.14 as the prevalence of sickle cell disease in pregnancy in the health center. It is therefore estimated that 1 out of every 714 women seen in our antenatal clinic in the region will have sickle cell disease. This is relatively lower than the prevalence of sickle cell disease in the normal population where it is estimated to be one in every 500 normal persons. Although 52 sickle cell disease in pregnancy were documented from this study over a-10-year period, this was higher than that documented by Ocheni, et al (2007) in his retrospective study in South-eastern Nigeria, where 10 sickle cell disease in pregnancy were documented over a period of 30 years. On the other hand, this was relatively lower than that documented by Odum et al (2002) in which 60 sickle cell disease in pregnancy were documented within 3-year period of retrospective study in South-Western Nigeria.

The study showed that over 57% of the booked patients in the clinic had tertiary level of education. This shows that female education has a role to play in creating awareness for maternal care. There is, therefore, need to empower women through education. This could be a strategy in the right direction of reducing maternal mortality in our environment. The 0.2% reflects that women with low level of education do not seek comprehensive care but resort to traditional birth attendants. Although, patterns of level of education of the sickle cell disease patients were not captured, future studies will look at these demographic parameters.

The average gestational age of 29.18 weeks at booking from this study indicate late booking. This means that majority of the women registered at the third trimester. This was relatively similar to the gestational age (24.33 weeks)
documented at booking in Abakiliki, South-eastern Nigeria. In this study it was found that 83.1% of the pregnant women booked after first trimester. This may not be in the interest of the maternal and fetal well-beings. Antenatal care is one of the pillars of SAFE Motherhood Initiative aimed at preventing adverse pregnancy outcome. Early antenatal booking is recommended for this benefit. When a woman books late in the ante-natal clinic, the benefit of safe motherhood is defeated. The advocacy has always been early booking as the panacea for favorable outcome. Only very few registered at the late first trimester. Although, this study could not capture the gestational age at booking for sickle cell disease patients, future study will look at pattern and determinants of antenatal booking in sickle cell disease patients.

Anemia in pregnancy used in this study was based on World Health Organization definition as Hemoglobin concentration less than 11 g/dl. This study showed that 39.3% of the patient presented with hemoglobin value less than 11.0 g/dl. This showed that more than one-third of the women were already having anemia at booking. This was similar to the values obtained in previous studies in Nigeria. Anemia in pregnancy is very common in low-and middle-income countries. 40 (77%) out of the 52 women with sickle cell disease had anemia which is one of the greatest burden of sickle cell disease in pregnancy. Pregnant women with sickle cell anemia are classified as high risk. This is because sickle cell disease increases the risk of certain complications such as miscarriage, hypertension, premature death just to mention a few. Similarly, the risk of sickle cell anemia to the unborn baby is quite enormous. It can lead to low birth weight, intra-uterine death (IUD), and abnormal presentations. In a study in Lagos, it was found that antenatal and postpartum blood transfusion rates for the sickle cell disease patient were 45.0% and 81.6% respectively. Anemia, evidenced by low hemoglobin concentration could be a predictive marker of women who may require blood transfusion during pregnancy or postpartum period.

One of the challenges facing sickle cell disease globally is underfunding and lack of publicity. Sickle cell disease is the single most common life-threatening genetic disease. Racial disparities come into play in private and public funding of sickle cell disease (believed to be commoner in black race) compared to other genetic disease such as cystic fibrosis (which are commoner in Caucasians) in high-income countries such as U.S.A. For example, the National institutes of Health (NIH) spends nearly four times as much per patients on cystic fibrosis research as it does on sickle cell disease despite the fact that four times the number of people suffer sickle cell disease as those with cystic fibrosis. The cost of managing sickle cell disease is so enormous that it is unprecedented, yet it is less funded and less advocated.

Sickle cell disease is associated with higher childhood mortality in low-income countries compared to high-income and some middle-income countries. With the current advances in the management of sickle cell disease commoner in high and middle-income countries, the average lifespan of people living with sickle cell disease has improved up to 3-4 decades of life, hence, a transition from mortality to morbidity (burden) of the disease. The implication is that more funds will be channeled towards managing the sickle cell disease crises and obstetric complications of sickle cell disease in pregnancy. Poor funding leads to poor motivation of the facilitators of sickle cell disease research projects. Most projects in Nigeria are funded by donor agencies and they are not readily available. The few available help partners do not have enough financial strength to drive sickle cell disease projects in the country. In addition, the lack of political will on the side of the government to own up research projects on sickle cell disease or to improve budgetary allocation to health sector has further worsen the care of people living with sickle cell disease in Nigeria. In addition, there is poor health information management system (HIM) in most health centers in Nigeria. This could lead to prolong turn-around-time in information retrieval system. In general, the atmosphere for research is impoverished, evidenced by under-developed infrastructure and absence of national policies for public health planning for people living with sickle cell disease.

Sickle cell disease is a preventable disease and so our strength to control this disease in sub-Saharan Africa is hinged on advocacy, communication and social mobilization (ACSM). This form the conceptual framework for the strategy in carrying out this study. Advocacy in this context simply connotes deliberate process of influencing those who make policy decisions. It is delivering messages that are intended to influence the actions of policy makers. There is poverty of knowledge about sickle cell disease and its impact in the vulnerable group (i.e. pregnant
mothers and under 5 children) in sub-Saharan Africa. As a result of this, many of them die due to complications which, under normal circumstances, would have been circumvented. Advocacy will create the awareness and send messages across the appropriate quarters for appropriate interventions to curb this menace. In order to drive sickle cell disease research project successively in Nigeria, a team of leaders with political, core transformational, trans-organizational and team competencies are needed. Advocacy relies on communication strategies to achieve its goals. Social mobilization relies on communication strategies to ensure community engagement.[20] The ultimate goal is to bring about the desired change which will improve the quality of lives of people living with SCD. In addition, viable health information management system (health informatics) evidenced by proper medical records with detailed demographic information of the participants, geographical information system (GIS), reliable data collection system, ethical clearance, community support and a team spirit are the strengths that will drive sickle cell disease projects to ensure qualitative implementation of the strategies.

In conclusion, sickle cell disease in pregnancy is a public health challenge. There is need to create the awareness through pervasive media networking, involving the government and other donor agencies in order to institutionalize appropriate health interventions aimed at early detection and treatment. This will impact positively on the quality of lives and pregnancy outcome of sickle cell disease mothers. Female education and empowerment should be regarded as topmost priorities in order to achieve this Millennium Development Goal-4 (MDG-4). There should be a national policy and budget to scale up the care and support of sickle cell disease in pregnancy in Nigeria. These strategies, if implemented, will go a long way in reducing the burden of sickle cell disease in our society.

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INTRODUCTION

A woman with sickle cell disease developed acute myelogenous leukemia with a background of myelodysplastic syndrome after seventeen years of treatment with hydroxyurea (HU). Whether this represents a chance association or reflects a leukemogenic risk of hydroxyurea cannot be determined. Even if such a long-term risk were to exist, it would be small and does not outweigh the immediate life-saving effect of hydroxyurea in sickle cell anemia.

CASE REPORT

Our patient is 71 years-old, has homozygous hemoglobin SS disease, started taking hydroxyurea at doses ranging from 1000 mg to 1500 mg per day since 1995, which she continued for 17 years. At age 60, she developed a hypoproliferative anemia associated with mild renal impairment and was treated with erythrocyte stimulating agents (ESA) for 11 years. She developed pancytopenia in 2012 which persisted despite discontinuation of hydroxyurea for more than three months. She also became transfusion dependent.

Laboratory results showed a WBC of 1.5 x 10^9 /µL, an absolute neutrophil count of 0.3 x 10^9 /µL, hemoglobin of 4.8 gm/dL, platelet count of 120 x 10^9 /µL, and absolute reticulocyte count of 43 x 10^3 /µL. Bone marrow core biopsy revealed 60-70% cellularity with 50% myeloblasts (Figure 1). There was dyserythropoiesis and dysmyelopoiesis on the aspirate (Figure 2). The myeloblasts were positive for CD 13, 33, 34, HLA-DR and MPO. A diagnosis of acute myelogenous leukemia with minimal differentiation (AML-M1) was made (1). Marrow cytogenetics were complex as shown in Table 1, with 43, XX karyotype, with 17p-, 5q-, 7q-, 12p- and other chromosome aberrations involving chromosomes 9, 20 and 22 in 70% of metaphases.

The patient also had chronic renal failure stage 3, iron overload, systemic and pulmonary hypertension, gout and hypothyroidism. After the diagnosis, prognosis, and management options were discussed, the patient opted for palliative care only. She died about a year after her AML diagnosis and permission for an autopsy was not granted.

CONCISE LITERATURE REVIEW

Table 1 lists all case reports of acute leukemia in sickle cell patients with (8 cases) and without (16 cases) exposure to hydroxyurea (2-7). The duration of hydroxyurea exposure ranged from two to fifteen years. Two patients took HU for less than 3 months and are listed among the unexposed cases. Such an interval is much too short for potential leukemogenesis. In addition to our case, two of the exposed patients had cytogenetic abnormalities. One had a complex composite karyotype of 44 chromosomes with a recurring unbalanced rearrangement of chromosome arm 5q, deletion of chromosome arm 7q, loss of chromosomes 15 to 22 and Y, and 5 marker chromosomes, suggesting a myelodysplastic syndrome or AML (6). The other had a 42XY with complex cytogenetics including, t (5;18), del(7)(q21) and −17 (7).

Case reports in unexposed patients cover about a 50-year period. In comparison, the seven HU-exposed cases were published within the last fourteen years corresponding, necessarily, to the period after hydroxyurea was approved for sickle cell disease treatment. The paucity of AML case reports in unexposed patients after 1986 may simply reflect a bias for reporting potential hydroxyurea-AML associations in sickle cell patients.

DISCUSSION

The beneficial effects of HU have been documented by randomized clinical trials in both adults (8) and young children (9). Recently, several published articles have
provided evidence that hydroxyurea can protect against or even reverse chronic organ damage (9-13). Other longitudinal studies strongly suggest that hydroxyurea improves patient survival both in adults (14, 15) and children (16) with sickle cell disease. There is reason to expect that an increasing number of adults and children with sickle cell anemia will be prescribed this drug. Nevertheless, hematologists/oncologists list carcinogenicity as one of the barriers to the wider use of hydroxyurea in sickle cell patients (17).

A Johns Hopkins review of toxicity of hydroxyurea in adults and in children with sickle cell disease suggests that there is no increased risk of leukemia from hydroxyurea (18). Another review strongly encourages widespread utilization of hydroxyurea among adults because of its efficacy (19).

The MSH investigators published a seventeen-year follow up of their study cohort and found no evidence of increased risk of malignancy (15). In non-SCD patients, therapy-related acute leukemia (t-AML) has a prevalence of about 0.2%, a 4.7 fold higher risk than in the general population (20). However, among younger adults t-AML risk is probably about 10-fold greater than in the general population (20). Our case is interesting also because of the additional eleven-year exposure to ESAs to reduce the number of transfusions for her severe anemia. Although ESAs are not considered to be leukemogenic, studies have shown that ESAs can worsen the clinical course of solid tumors. There are isolated reports associated with the development of monoblastic leukemia arising from MDS (21) and acute leukemia with a uremic patient (22) with the use of ESA treatment.

We believe that in sickle cell disease the benefits of hydroxyurea are immediate and outweigh a potential low and late leukemia risk. Physicians need to strongly encourage the use of hydroxyurea in this patient population. We also believe that the unknown risk of leukemia with hydroxyurea should be discussed with the patients and their families.

REFERENCES


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