Vaso-occlusion in sickle cell anemia

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Introduction

Sickle cell anemia (SCA) is a common inherited congenital hemolytic anemia affecting millions of persons worldwide. SCA is characterized by a predominance of abnormal sickle hemoglobin (HbS) within the erythrocytes, which polymerizes under deoxygenating conditions, leading to deformed (sickled) cells with increased adhesiveness, aberrant membrane composition, and cellular dehydration. These intrinsic defects, coupled with abnormal flow characteristics of sickled erythrocytes, lead to erythrocyte fragility and pathological interactions with the endothelium and reticuloendothelial system; hence, the SCA is a hemolytic anemia with both an intravascular and extravascular component.

SCA is also a chronic inflammatory condition featuring widespread endothelial damage, dysfunction, and activation. The vasculopathy of SCA is now well-characterized but still not completely understood. The intravascular milieu features sickled erythrocytes, adhesive circulating blood cells, hypercoagulability, plasma mediators of inflammation, and products of hemolysis. Together, these factors promote abnormal cellular attachments and interactions with the endothelium, which then alter normal vessel integrity and lead to pathological responses. The processes by which circulating leukocytes, reticulocytes, and sickled erythrocytes adhere to vascular endothelium and the effects of these interactions are increasingly understood using *in vitro* and animal models, which provide an opportunity to discover and test therapeutic interventions.

Clinically, patients with SCA have periodic acute events that reflect abrupt blockage of blood flow within small vessels, leading to distal tissue hypoxia and infarction; this process is usually referred to as "vaso-occlusion", since normal blood flow is impaired. These sporadic events are typically painful and often require medical attention, while some present mainly with respiratory or neurological symptoms. This sickle cell *crisis* is among the more perplexing aspects of SCA, and its optimal clinical management is the subject of numerous current clinical trials. This review focuses on vaso-occlusion in SCA, which refers predominantly to patients with homozygous HbSS. Although patients with other genotypes, such as HbSC and HbS/thalassemia, also have vaso-occlusive events, their pathophysiology is different and less studied and will not be emphasized further in this review.

Nomenclature

The nomenclature surrounding the pain that is so characteristic of SCA has recently been called into question regarding cultural sensitivity and appropriateness. Although the origin and first use of "crisis" for SCA patients is unknown, this word is quite useful and has long been accepted, since it encompasses both the suddenness and severity of the pain. Particularly in discussions involving the lay community, the word is common but can be preceded by descriptors such as "pain" or "painful" or even "acute" to accentuate the symptoms. Among healthcare providers, the word "vaso-occlusive" is added to emphasize the pathophysiology of the pain and to distinguish it from other etiologies, and "vaso-occlusive crisis" has become the recognized VOC acronym. Subsequently, however, the terms "event" and "episode" have been advocated over "crisis", since these are potentially less pejorative and remove any suggestion of psychological overlay or origin. Phrases like "vaso-occlusive event (VOE)" or "painful event" have thus become commonly used to describe SCA pain that requires medication or medical evaluation. The fact that a 2011 sickle cell conference had a highlighted topic titled "Don't take away my Crisis" that features a debate on the merits and sanctity of the word "crisis" in SCA indicates that the issue is still far from resolved. In an attempt to remain neutral yet informative, the current text will simply use the descriptive but admittedly rather bland term "vaso-occlusive event" to describe these sickle-related pain and other acute processes.

Pathophysiology

The topic of sickle cell vaso-occlusion has been extensively studied over the past 40 years, and our understanding of the process has evolved considerably since the original simplistic concept of misshapen sickled ery-

throcytes forming a transient log-jam within small vessels, thereby impeding local blood flow until released. One major advance came 30 years ago when several investigators identified abnormal adhesion between sickle erythrocytes and vascular endothelium,[1-2] indicating that deoxygenation and HbS polymerization had effects beyond changing erythrocyte shape. Elucidation of the critical role of circulating leukocytes in triggering this contact was another major step forward.[3-4] Several recent reviews have highlighted the numerous advances and refinements of the current state of knowledge about vaso-occlusion in SCA since that time.[5-6] Vaso-occlusion still begins with sickled erythrocytes but is now known to be a highly complex process involving frequent abnormal interactions among a wide variety of circulating blood cells, plasma factors, and vascular endothelium. Each major component of this triad contributes to the vaso-occlusive process and the interactions are circular, highly interactive, and often amplifying (Figure 1). Proper understanding of vaso-occlusion, with consequent blockage of blood flow and distal tissue hypoxia, requires recognition and discussion of all aspects of the pathophysiology within this triad.

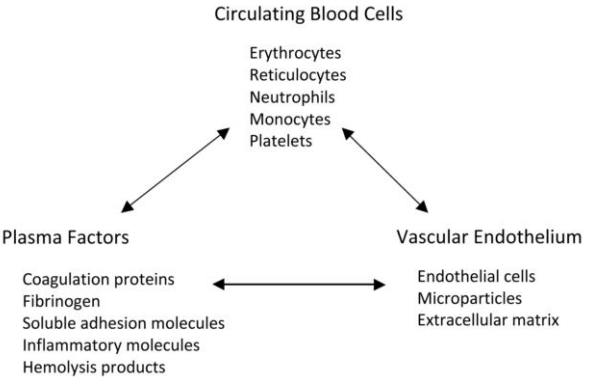

Figure 1. Main blood components participating in the complex interactions of vaso-occlusion in SCA. Although the sickling process begins within erythrocytes, many other circulating blood cells become involved as primary and secondary participants. Plasma factors promote vaso-occlusive and reflect cellular damage and vascular inflammation. The vascular endothelium is progressively damaged and its inflammation and dysfunction leads to additional vaso-occlusion and vasculopathy. These interactions are circular and amplifying.

First and foremost, it should be emphasized that the vaso-occlusion event observed in SCA is unique and does not have another medical equivalent; this fact mandates that the pathophysiology begins and hinges upon the presence of HbS and the sickled erythrocyte. Abundant HbS within the erythrocytes, which leads to intracellular polymerization, morphological shape changes, and abnormal cell-vessel interactions, cannot be overlooked as the principal culprit of the vaso-occlusive process. Newborn infants with SCA have low amounts of HbS (typically 5-25%) and appear to be protected in the early neonatal period due to high amounts of intracellular fetal hemoglobin (HbF). Vaso-occlusive events do not develop clinically until the protective effects of HbF begin to wane, usually in the first few years of life.[7] Conversely, hereditary persistence of HbF provides lifelong protection against clinical events,[8] which further emphasizes the critical importance of the erythrocyte (and its HbS concentration) in the genesis and development of vaso-occlusive events. The sickle erythrocyte is not simply an inert carrier of HbS, however, because the erythrocyte membrane itself is abnormal[9] and coated with adhesive molecules that promote adhesion to the endothelium. Reticulocytes are especially adherent,[10] perhaps because they are less dense and retain surface markers from the marrow that are not removed or "polished" in the absence of a healthy spleen. Sickle erythrocytes express a wide variety of proteins and lipids, including exposed phosphatidylserine, which aberrantly bind to vascular endothelium and the subendothelial matrix as a critical part of the development of vaso-occlusive events.[11-16]

Over time and after repeated abnormal sickled erythrocyte-vessel interactions, these events damage the vascular endothelium and begin a slow but almost inexorable slide toward generalized endothelial injury, dysfunction, and activation. Damaged endothelium cannot properly produce local nitric oxide or detoxify reactive oxygen species, which leads to further endothelial injury through hypoxia/reperfusion events.[17] Circulating endothelial cells (CEC) can be identified in SCA,[18] often with an abnormal surface marker profile reflecting widespread vascular damage. Levels of CEC are elevated in steady-state SCA but further increase during acute vaso-occlusion and may reflect disease severity.[19] Microparticles derived from endothelium also circulate in SCA.[20] Activation markers can be detected on these circulating cells and microparticles, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, P-selectin, and tissue factor.[21-24] Not all endothelium is identical, however, and it is likely that intrinsic differences in the vascular endothelial beds themselves are important determinants of the cerebrovascular and pulmonary damage in SCA that begins early in life and could perhaps be as important as physiological fluctuations such as blood flow and oxygenation. This is an area that has not been fully explored but could reveal insights into the pathophysiology of vaso-occlusion and help explain the propensity for damage within certain organs.

Another critical consequence of these abnormal erythrocyte-endothelial interactions is the progressive development of a generalized inflammatory process. As previously mentioned, circulating erythrocytes, and especially younger reticulocytes, have increased levels of adhesion and activation molecules. The total white blood cell (WBC) count becomes elevated early in life,[25] particularly an elevated neutrophil count, along with increased platelets, and these circulating cells feature surface adhesion markers that reflect activation and promote adhesion to endothelial cells.[26-27] Activated $CD11b^+$ monocytes have also been identified in SCA; these circulating cells stimulate endothelium through TNF-alpha and interleukin-1-beta and trigger nuclear factor-kappa B nuclear translocation,[28] which then presumably leads to further endothelial activation. Each of these cells also spawns microparticles that express inflammatory and activation markers.[20,29] Within the plasma itself, a wide variety of soluble non-specific inflammatory markers can be identified, including elevated levels of C-reactive

protein, cytokines, and soluble adhesion molecules like ICAM-1, VCAM-1, and E-selectin.[30-31] Taken together, it is clear that SCA features widespread and chronic inflammation that affects circulating cellular elements, cellular-derived microparticles, and soluble plasma factors that collectively promote vaso-occlusion and endothelial damage.

An additional characteristic feature of SCA that is likely important in vaso-occlusion and organ damage, but relatively poorly understood at this time, is the hypercoagulable state.[32] A variety of factors could contributes to hypercoagulability in SCA, including functional asplenia, reticulocytes and erythrocytes that express adhesion molecules and exposed surface phosphatidylserine, increased numbers of WBC that express increased adhesion markers, activated platelets,[30,33] chronic inflammation, and elevated levels of plasma clotting proteins.[34-35] Owing to a combination of these abnormalities, the coagulation system in SCA is clearly tilted toward the procoagulant state. Whether or not this hypercoagulability initiates cell-endothelial contacts remains unclear, but almost certainly it promotes and propagates the pathophysiology of vaso-occlusion. It should be noted, however, that patients with SCA do not develop deep venous thrombosis or other common manifestations of a hypercoagulable state.

Finally, the contributions of products of hemolysis toward vaso-occlusion must be considered in the unique setting of SCA. Sickled erythrocytes are fragile, and about one-third of the overall hemolysis in SCA occurs in the intravascular compartment.[36] Considerable effort has been directed toward promoting the hypothesis that this intravascular hemolysis, measured specifically by elevated levels of plasma hemoglobin but more often by lactate dehydrogenase (LDH), is itself a direct and critical etiologic factor of vascular endothelial damage in SCA through consumption of nitric oxide, with substantial clinical consequences, including stroke, leg ulcers, pulmonary hypertension, and early mortality.[37-38] However, the merits of these arguments have been recently questioned with regard to their scientific rigor and accuracy.[39-40] Indeed, many other hematological disorders include intravascular hemolysis with similarly elevated levels of LDH (or higher), yet none features the clinical spectrum of SCA. The most likely answer to this apparent paradox is that no other medical condition features intravascular hemolysis so prominently in the setting of generalized inflammation, hypercoagulability, and endothelial dysfunction;[39] hence hemolysis per se is not the etiology of the vasculopathy in SCA but merely a participant. Another alternative explanation regarding hemolysis-based pathophysiology warranting further experimental testing is the possibility that some elevated serum LDH in SCA actually derives from damaged and/or circulating endothelial cells rather than just from erythrocytes; if proven, this observation would suggest that elevated LDH reflects an "effect" rather than a "cause" of the endothelial damage in SCA. Additional possibilities should be explored as well, including the recent proposal that hemolysis-derived pathophysiological characteristics of SCA, such as excessive plasma heme, also contribute to blood cell and endothelial damage.[40-41]

In this complex environment, the process of small vessel vaso-occlusion in SCA thus begins initially with repeated erythrocyte sickling and adhesion-mediated vessel blockage, but over time develops into a unique vasculopathy characterized by activated circulating blood cells, including erythrocytes, reticulocytes, neutrophils, monocytes, platelets, and even endothelial cells. These sticky cells are then coupled with soluble plasma mediators of inflammation, coagulation, and hemolysis, which lead to further adhesion that alters the normal integrity of the endothelium. Perhaps in this setting of such widespread cellular adhesion, damage, and inflammation, the question should not be "Why do patients with SCA have acute vaso-occlusive events?" but instead "Why do patients with SCA not have vaso-occlusive events all the time?" since the microvascular processes are both widespread and continuous. It should be emphasized that vaso-occlusion is ongoing even when not clinically recognizable.

Epidemiology

Most of the data regarding the types and frequency of acute vaso-occlusive events in SCA derive from two large prospective studies, the Jamaican cohort and the US multicenter Cooperative Study of Sickle Cell Disease (CSSCD). These studies have documented that painful events, acute chest syndrome, and stroke are frequent clinical manifestations that lead to substantial morbidity and mortality.[42-44] However, there is considerable phenotypic diversity with regard to vaso-occlusive events; most patients are affected occasionally, but a small percentage of patients suffer from frequent and repeated events.

In the first decade of life, pain occurs at a surprisingly low average frequency of events per year, based on recall of pain severe enough to warrant medical evaluation.[7] Hand-foot syndrome (dactylitis) occurs primarily under age 2 years, while long-bone pain is more common among older children and adults.[43,45] Acute chest syndrome in young patients can have an infectious component but becomes exacerbated by intrapulmonary sickling and vaso-occlusion.[46] Stroke is perhaps the most severe form of acute vaso-occlusion in children with SCA,[47] featuring abrupt interruption of cerebral blood flow, with consequent damage to both grey and white matter.

Among teens and adults with SCA, pain remains a serious and occasionally debilitating part of their disease. A recent multicenter study has documented that sickle-related pain, both acute and chronic, is typically under-reported and diminishes quality of life.[45,48] Acute chest syndrome from acute vaso-occlusion becomes more frequent and more dangerous, often escalating into a life-threatening process characterized by respiratory distress with hypoxia, acute pulmonary hypertension, multi-organ failure, and death.[46] Stroke occurs in adults but is more often hemorrhagic than vaso-occlusive in etiology.[47] In adulthood, chronic vaso-occlusion (or repeated acute events) becomes equally as important as the acute clinical events, with progressive damage to the kidneys, brain, eyes, bones, and other organs that ultimately leads to early mortality.[44]

Prediction

Perhaps the most characteristic feature of acute vaso-occlusion in SCA is its unpredictability of onset and severity. Often the acute episode arises from a relatively stable steady-state. Some painful events have recognizable physiological and environmental triggers, such as dehydration, change in temperature or weather, and infection, but for most patients and most events, there is no identifiable trigger at all. Patients with previous acute vaso-occlusive events tend to have more future events, but that observation is hardly useful for an individual patient. Owing to the remarkable diversity in how vaso-occlusion develops and presents, a better understanding about predicting vaso-occlusive events remains a worthwhile yet elusive goal.

Data from the prospective Cooperative Study of Sickle Cell Disease indicate that low HbF and elevated WBC are the two most important laboratory parameters that influence the number of acute vaso-occlusive events and overall clinical severity.[43-44,49] HbF presumably exerts its powerful influence through lowering intracellular HbS concentration and inhibiting polymerization; the simple phrase "more is better" summarizes the benefits of higher HbF levels. The elevated WBC count is presumably a result of inflammation and cellular damage, but these adhesive leukocytes can also contact endothelium and initiate vaso-occlusion.[50] For these reasons, the WBC should be considered "cause-and-effect" with regard to being a risk factor for acute vaso-occlusive events. It should be noted, however, that a recent large prospective cohort study failed to validate previously published predictors of vaso-occlusive events and clinical severity among children with SCA.[51]

Genetic modifiers have become popular as a potential explanation for the clinical diversity observed in SCA. Specific genetic polymorphisms outside of the beta-globin locus might represent independent risk factors for the development of acute vaso-occlusive events.[52] In most cases, the validity of these results has not been established using independent patient cohorts; furthermore, the clinical utility of such genetic modifiers remains unclear, as well. It is possible that well-validated genetic modifiers of acute vaso-occlusion might eventually lead to a more personalized treatment plan for certain individuals with these polymorphisms, specifically one that encourages early and preventive treatment.

Treatment

The development and use of hydroxyurea for the treatment of SCA should be considered the most important therapeutic advance to date. Hydroxyurea cannot be used as a specific or urgent treatment for an acute vaso-occlusive event, however, so it will be discussed in the section on Prevention.

The time-honored therapeutic interventions for acute painful vaso-occlusive events include hydration, analgesia, and anti-inflammatory medications. Increasing the circulating blood volume helps open blocked capillaries and assists erythrocytes back to oxygenation. Hydration can be provided either by oral or intravenous means, depending on the severity of the event and the patient's ability to tolerate oral fluid intake. Maintaining mild hyponatremia (e.g., serum sodium ~135 meq/mol) provides a slight osmotic gradient favoring water entry into the circulating erythrocytes, thereby lowering their intracellular HbS concentration, but greater degrees of hyponatremia should be avoided. When the vaso-occlusive event involves the lungs, care must be taken to avoid over-hydration that can worsen acute chest syndrome and lead to pulmonary edema and effusions.

Analgesia can be provided using non-narcotic or narcotic medications, depending on severity, previous use, and tolerance of narcotics. Many patients use a combination of non-narcotics and narcotics as out-patient therapy, but the latter category is indicated when patients are hospitalized. Intravenous narcotics such as morphine or hydromorphone are optimally delivered using patient-controlled analgesia methods, which allow continuous infusion with small boosts as needed for improved clinical outcomes.[53] Anti-inflammatory medications such as non-steroidal compounds are commonly used to reduce the clinical symptoms of acute vaso-occlusion. Corticosteroids are effective at reducing inflammation but are often associated with rebound symptoms,[54-55] so are no longer routinely used in the setting of acute vaso-occlusion.

Additional commonly used interventions include incentive spriometry, heat, physiotherapy, or other forms of supportive comfort measures. Together these interventions provide some symptomatic relief but do little to alter the timing or natural course of the vaso-occlusive event. It should be noted that supplemental oxygen is not indicated for painful events unless there is concomitant hypoxemia; this common treatment measure is not warranted and could theoretically reduce the erythropoietic drive.[56-57] Similarly, blood transfusions are not helpful to relieve pain associated with acute vaso-occlusion.

More specific and even targeted therapy should be possible for the treatment of acute vaso-occlusive events, based on our current knowledge of the pathophysiological processes involved (Table 1). However, most investigations to date have been limited due to a relative paucity of testable compounds, lack of a robust clinical research infrastructure, and poor clinical enrollment. Especially for industry-sponsored trials, unrealistic demands for single-agent success have limited efforts to build an armamentarium of treatment options. For

Table 1. Pathophysiological processes involved in the vaso-occlusive event of SCA that might offer opportunities for non-specific or even targeted therapeutic intervention.

Blood Cells	Plasma	Endothelium
Sickling	Adhesion	Adhesion
Adhesion	Inflammation	Inflammation
Hypoxemia	Hypercoagulability	Vasculopathy
Oxidative Stress	Hemolysis	Reperfusion Injury
Hemolysis		Activation

example, improving blood viscosity could theoretically help alleviate acute vaso-occlusion, and published industry-sponsored Phase 1/2 results with polaxmer-188 (Flocor) were encouraging,[58] but no further Phase 3 investigation ensued. Similarly, a novel Gardos channel blocker that improves erythrocyte hydration showed promising cellular effects in a Phase 2 trial,[59] but the industry-sponsored Phase 3 trial was halted when it did not significantly reduce pain when tested as a single treatment modality. An industry-sponsored trial using inhaled nitric oxide was closed prematurely without release of results to date. Without industry backing, however, agents that are off-patent can suffer from lack of sponsorship and interest.

Therapeutic interventions directed against the adhesive properties on circulating leukocytes, endothelial cells, and microparticles offer an exciting new opportunity to treat acute vaso-occlusion. General inhibitors of adhesion such as intravenous immunoglobulin show efficacy in an animal model of SCA,[60] suggesting that interruption of cell-endothelial contacts may be therapeutic. Specific inhibitors of individual adhesion molecules also show promise,[61-62] especially a novel "panselectin" compound that should theoretically provide broad yet specific inhibition of leukocytes and other circulating cells.[63]

Targeted therapy for the endothelium itself reflects the observation that the chronic inflammatory vasculopathy triggers and participates in acute vaso-occlusion. Treatment to reduce the activation state of endothelial cells[24,64] has shown promise in animal models, and direct delivery of nitric oxide could restore vascular tone to help prevent vaso-occlusion.[65] Indeed, treatment of vaso-occlusion may require treatment for the endothelium as well as the erythrocyte,[66] since both are important in the pathophysiology. The idea of multimodal therapy for SCA has been proposed,[67] much like that often required for optimal therapeutic outcomes of treatment for infections or malignancies.

Open clinical trials

The large number of currently open therapeutic clinical trials for acute vaso-occlusion suggests that the treatment armamentarium may improve in the near future. A search for "sickle cell crisis" on the ClinicalTrials.gov website in January 2011 identified a total of 15 open therapeutic clinical trials (Table 2). A variety of Phase 1, 2, and 3 trials are currently enrolling subjects. Treatments range widely from specific (e.g., Adenosine 2A agonist, GMI-1070 anti-panselectin compound, eptifibatide, L-glutamine) to non-specific (e.g., IVIG, fish oil capsules) interventions. Some trials are being conducted in the US, while others originate from Europe; some are industry-sponsored, while others have governmental funding.

The ClinicalTrials.gov website also lists a large number of completed therapeutic trials for acute vaso-occlusion in SCA. While most results have not been provided on the website to date, interventions with widely diverse agents such as fructose, corticosteroids, ketamine, ketorolac, sodium nitrite, aspirin, magnesium pidolate, nitroglycerin, sildenafil, poloxamer 188, stem cell factor, varespladib, nitric oxide, ICA-17043 channel blocker, and far red infrared radiation have all been completed over the past few years.

Prevention

In light of the severe clinical consequences and morbidity associated with vaso-occlusion, as well as the chronic inflammatory state with endothelial dysfunction that develops as a result of repeated vaso-occlusion, it is perhaps obvious that prevention of the process would be preferable to acute intervention. Toward that goal, hydroxyurea is now a well-established oral agent with proven laboratory and clinical efficacy for SCA, ranging from infants to children to teens to adults.[68-71] The primary benefit of hydroxyurea derives from its

Table 2. Active registered clinical trials involving therapeutic intervention for patients with SCA and vaso-occlusive events, typically designed to treat acute pain in the emergency or inpatient setting. All trials registered at clinicaltrials.gov with open recruitment as of January 2011 are listed, along with two studies scheduled to open recruitment soon.

Clinical Trials #	Intervention	Phase	Current Status
NCT01085201	Adenosine 2A agonist	1	Recruiting
NCT01256281	Femoral nerve block	1	Recruiting
NCT00644865	IV immunoglobulin vs placebo	1,2	Recruiting
NCT01033227	Sodium nitrite, open label	1,2	Recruiting
NCT00834899	Eptifibratide vs placebo	1,2	Recruiting
NCT00142051	Inhaled nitric oxide vs placebo	2	Recruiting
NCT01119833	GMI-1070 (anti-panselectin)	2	Recruiting
NCT01197417	Magnesium sulfate vs placebo	2,3	Recruiting
NCT00748423	Inhaled nitric oxide vs placebo	2,3	Recruiting
NCT00999245	High vs Low demand narcotics	3	Recruiting
NCT00313963	Magnesium sulfate vs placebo	3	Recruiting
NCT01179217	L-glutamine vs placebo	3	Recruiting
NCT00874172	Nitrous oxide and nefopam vs usual	3	Recruiting
NCT01202812	Omega-3 fatty acids vs placebo	2	Recruiting soon
NCT00880373	Ibuprofen vs placebo	3	Recruiting soon

induction of HbF, yet it offers additional benefits through a wide range of mechanisms of action, including reduction in the number of circulating reticulocytes and leukocytes, improved erythrocyte shape and deformability, and even local NO production.[72]

Importantly for the pathogenesis of vaso-occlusion, the benefits of hydroxyurea in SCA have been reported for reducing adhesiveness of erythrocytes,[73-76] reticulocytes,[77] leukocytes,[78] and even endothelial cells.[79] Reduction in plasma endothelin-1, a marker of inflammation, has been measured,[80] as well as a number of cytokines, in a small number of patients.[81] While it is tempting to hypothesize that hydroxyurea has a broad salutary influence on adhesion and inflammation, a large prospective study is needed to test this formally. For example, a large panel of plasma inflammatory markers should be measured using newly available multiplex platforms, before any definitive conclusions can be reached about the impact of hydroxyurea therapy on the inflammatory pathophysiology of vaso-occlusion in SCA. A clearer understanding of these non-HbF effects of hydroxyurea will likely increase our enthusiasm for this drug and add to the argument that this potent once-daily oral agent should be offered to most, if not all, patients with SCA.[82]

Conclusions

The pathogenesis and pathophysiology of vaso-occlusion in SCA has been investigated for over 40 years, yet important questions still remain. With recent increased understanding of the complexity of vaso-occlusion, we now have opportunities for targeted therapies to interrupt the process; several key clinical trials with novel agents are currently underway. Ultimately, however, prevention of vaso-occlusion should be viewed as the optimal treatment outcome. Until a better agent or treatment regimen is developed, hydroxyurea should be offered more often and better utilized in this patient population.

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