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New Insights into Sickle Cell Disease: Mechanisms and Investigational Therapies

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Abstract

Purpose of review—Sickle cell disease (SCD) afflicts nearly millions worldwide. The simplicity of its single nucleotide mutation belies the biological and psychosocial complexity of the disease. Despite only a single approved drug specifically for the treatment of SCD, new findings provide the direction forward.

Recent findings—The last year has provided a wealth of support for mechanisms affecting the red cell, hemolysis and vasculopathy, the innate immune system activation, blood cell and endothelial adhesiveness, central sensitization to pain and chronic brain injury. The evidence supporting expanded use of hydroxyurea continues to mount. Many promising therapies are reaching clinical trial, including curative therapies, with more on the horizon.

Summary—Evidence is compelling that the use of hydroxyurea must be expanded by clinicians to gain the full pleiotropic benefits of this approved drug. Clinicians must become aware that severe acute and chronic pain has a biological and neurologic basis, and the understanding of this basis is growing. Researchers are testing investigational therapies at an unprecedented pace in SCD, and partnership between patients, researchers and the private sector provide the most rapid and productive way forward.

Keywords

Sickle cell; hemolysis; vasculopathy; inflammation; hydroxyurea

Introduction

New insights in the pathophysiological mechanisms of sickle cell disease (SCD) rise on the tide of new published research. The new millennium has seen a remarkable acceleration in basic, translational and clinical research publications in SCD. Since the year 2000, the output of peer-reviewed publications indexed in PubMed has risen by an average of 50 per year (Fig. 1), rising from an average around 400 per year to nearly 1200 publications in 2015. Space limitations will allow only a fraction of the most significant new publications in

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Disclosures: Hydroxyurea is not approved for use in children. IVIG is not approved for use in sickle cell disease. Rivipansel is an investigational agent. DEANO is a research reagent. Dr. Kato has served as a paid consultant or received research funding from AesRx, LLC, Baxalta, Biogen Idec, CSL Behring and Bayer.

2015 to be reviewed here, and many fine articles will be omitted here. This has been accompanied by a quite remarkable growth in pharmaceutical research in SCD [1], as well strides in clinical research and coalescence of evidence-based clinical practices [2]. This review will focus on the highest impact print and electronic publications of 2015 involving the pathophysiology of SCD, and will summarize the mechanistic and clinical practice insights from these publications.

Red Cells in SCD

Over a century of research publications have characterized the pathological effects of sickle hemoglobin upon the physiology of the red cell. Scientific observations in 2015 reinforce and solidify previous findings, and in some cases, provide unexpected new important insights about altered physiology of sickle erythrocytes. A genetic screen of randomly mutated mice uncovered a new activated mutant of the erythroid K-Cl cotransporter Kcc1. When bred to a sickle cell mouse strain, the excessive Kcc1 activity amplified the severity of SCD, with accumulation of extensive tissue damage and earlier mortality [3]. These data support longstanding biochemical evidence of Kcc1 dysregulation of red cell ion balance in SCD, and more broadly, the contribution of ion balance dysregulation to cellular dehydration that concentrates sickle hemoglobin, promoting its polymerization, the root cause of SCD pathology that promotes vaso-occlusion, tissue hypoxia and hemolysis [4]. These mechanisms in turn promote recently identified adenosine signaling via the adenosine A2B receptor on red cells to activate sphingosine kinase-1 activity to produce sphingosine-1-phosphate, which appears to bind directly to hemoglobin to stabilize the T-conformation of sickle hemoglobin that has lower oxygen affinity and promotes further polymerization [5].

Persistent expression of fetal hemoglobin is the best-documented mechanism to reduce polymerization of sickle hemoglobin and all features of SCD. Inhibition of BCL11A relieves its repression of fetal hemoglobin in erythroid development, but a recent study of deletions in the BCL11A promoter identified neurological impairment as an undesired consequence of its upregulation if fetal hemoglobin. These results guide future gene editing strategies for possible gene therapy of SCD [6]. In the meantime, other investigators continue to identify new targets and small molecules that stimulate expression of fetal hemoglobin. These approaches include inhibition of G9a methyltransferase [7], inhibition of EHMT1 and EHMT2 histone methyltransferases [8], inhibition of lysine-specific demethylase 1 (LSD1) [9], and novel small molecule inducers of fetal hemoglobin identified in high-throughput screening [10, 11]. The robust efforts by many investigators to boost fetal hemoglobin expression using a wide variety of strategies represents the progress gained from long term investment in funding this field, raising promise for future therapeutics, but the pathway to drug development in any field is always long.

Non-intuitively, new evidence has arisen that supports iron restriction as a potential strategy for to improve the anemia of SCD. Transplantation of sickle cell mouse bone marrow into recipient mice with genetically reduced intestinal iron absorption resulted in lower serum and tissue iron content, leading to markedly less severe hemolytic anemia [12]. Seemingly paradoxical, this finding is consistent with alterations in iron trafficking in the thalassemia mouse that improve ineffective erythropoiesis, a little appreciated aspect of SCD. Isolated

case reports have hinted that iron restriction might follow the mechanism of alpha thalassemia trait in SCD, which limits intracellular hemoglobin concentration, thereby reducing polymerization of sickle hemoglobin and reducing hemolysis. Manipulation of iron trafficking in SCD merits additional investigation.

Hemolytic Anemia, Nitric Oxide and Vasculopathy

Patients with SCD with more severe hemolytic anemia have a higher prevalence of several specific complications of SCD, observed in independent epidemiological studies, too numerous to detail here. Severely anemic SCD patients have higher risk of developing pulmonary hypertension, ischemic stroke, leg ulcers and priapism. These complications characterized by impaired vasomotor control and proliferative changes in the affected blood vessel wall when they occur in both the SCD and general population. This complex field can't be reviewed comprehensively here, but new data from 2015 more closely characterize the outcomes and physiological pathways related to pulmonary and systemic vascular disease in SCD (Table 1) [13].

Cardiac magnetic imaging shows that SCD patients with a higher transpulmonary gradient (TPG>12mmHg), a simple right heart catheterization measure of pulmonary vascular disease, develop prominent remodeling of the right ventricle and reduced right ventricular ejection fraction consistent with high pulmonary artery pressure, and these variables predict reduced exercise tolerance and earlier death, very analogous to pulmonary hypertension in the non-SCD population [14]. These results indicate the adverse impact of pulmonary vasculopathy on the heart, and strongly suggest a relationship to exercise capacity and death. This conclusion was further supported by a new meta-analysis of pulmonary artery pressure estimated by echocardiography [15]. This meta-analysis of 45 studies totaling 6109 patients across 15 countries found higher than normal estimated pulmonary artery systolic pressure (ePASP) in 21% of children and 30% of adults with SCD. Patients with elevated ePASP had reduced exercise capacity and earlier mortality (hazard ratio of 4.9 (2.4-9.7)). The association of invasive and noninvasive measures of pulmonary vascular disease with impaired functional capacity and death is strongly upheld by these meta-analysis results from 2015, even though small studies are each able to document only parts of this big picture [16].

New research supports a link between pulmonary and systemic vasomotor dysfunction in SCD. Tricuspid regurgitant velocity (TRV), the indirect echocardiography measure of pulmonary artery pressure used to calculate ePASP, was shown to correlate significantly to systemic vasomotor function measured by flow-mediated dilation (FMD), a non-invasive ultrasound measure of endothelial dysfunction in the brachial artery in children with SCD [17]. These indicators of pulmonary and systemic vasculopathy each showed striking correlation to free hemoglobin in plasma, a gold standard marker of intravascular hemolysis. Intervention with transfusion therapy, which improves the severity of both anemia and hemolysis, partially corrected the abnormal FMD in SCD patients; and chronic transfusion therapy was associated with protection against both pulmonary and systemic vasculopathy in this cohort. The role of free hemoglobin in NO scavenging was upheld by evidence that acute hemolytic vascular inflammatory processes are prevented by NO replacement or a

single dose of hydroxyurea [18], which has been well-demonstrated in the past to be an NO donor.

A growing theme in SCD is the role of excess free heme, which is released from free hemoglobin that has been oxidized or denatured, already shown to promote vaso-occlusion in SCD. Free heme in excess promotes tissue factor-dependent activation of coagulation in mice, and may contribute to the prothrombotic state observed in SCD [19]. Microvesicles generated from erythrocytes in SCD contain hemoglobin and free heme, which they can transfer to endothelium, and promote vaso-occlusion in sickle cell mice, in particular targeting the kidney [20]. Indeed, SCD patients with higher grade intravascular hemolysis accumulate more iron in the kidney, and this is associated with albuminuria [21]. Chronic renal injury with albuminuria and hemoglobinuria is more evident in SCD patients with genetic variants compromising protective functions of the hemoglobin scavenger apolipoprotein L1 or the heme catabolism enzyme heme oxygenase-1 [22], emphasizing the contribution of chronic intravascular hemolysis in the development of sickle nephropathy.

The steady increase in evidence implicating hemolysis in the development of organ dysfunction in SCD highlights the need for therapeutic strategies that protect against free hemoglobin and free heme. Haptoglobin is a normally highly abundant plasma protein that binds hemoglobin and carries it to the CD163 receptor on monocytes, macrophages and dendritic cells. New animal model data show that haptoglobin forms complexes with hemoglobin that are too large to penetrate endothelial cell-cell tight junctions, thereby keeping hemoglobin out of the subendothelial space where its NO scavenging activity presents the greatest vasculotoxic injury [23]. These results point to a potential role for therapeutic haptoglobin supplementation in disorders of intravascular hemolysis, in which haptoglobin becomes depleted as it escorts hemoglobin into monocytes and macrophages.

An alternative strategy invokes the heme scavenger protein hemopexin. In sickle cell mice, heme skews the macrophage repertoire toward an M1 pro-inflammatory phenotype. Hemopexin injection prevented this macrophage skewing, as hemopexin appeared to redirect free heme from the macrophages to hepatocytes, which appear to detoxify the heme [24]. Which is the better strategy to translate to potential future therapies? Additional data support a concept that the combination of haptoglobin and hemopexin already designed by nature provides a more complete vasculoprotective package against the toxic products of hemolysis [25]. The area of haptoglobin-hemopexin replacement therapy is promising and gaining attention. The new findings of 2015 fit into a hypothetical model of interactions summarized in Figure 2.

Brain and Pain Campaign

Young children with sickle cell disease have a remarkably high risk of ischemic stroke, with a reverberation in late adulthood. 2015 was a year of advancement in understanding stroke prevention, the more subtle brain manifestations of SCD and the dysregulation of the central and peripheral nervous system that accompany the characteristic pain of SCD (Fig. 3). The risk of stroke in children with SCD has been nearly eliminated by monthly red cell transfusion therapy in high-risk children identified by high velocity blood flow in their

cerebral arteries detected by noninvasive transcranial Doppler ultrasound. New data indicate that chronic and acute anemia, desaturation and carotid artery stenosis are risk factors for silent cerebral infarcts and white matter injury in sickle cell [26, 27]. Furthermore, silent cerebral infarctions before age 5 years predicts progressive MRI abnormalities, cerebrovascular stenosis, cognitive abnormality, poor academic performance and 56% risk of overt stroke or transient ischemic attack [28]. The total volume of white matter injury predicts the extent of neurocognitive dysfunction [29]. Boys seem to be at highest risk, and alterations in circulating von Willebrand protease activity predicts deficits in cerebral blood flow, suggesting a relationship to vasculopathy [30]. Examination of finer neuroanatomical structures in the brain with 7 Tesla MRI scans discloses cortical hyperintensities, and in adults, venular rarefaction with shortening of venules, associated with cognitive impairment and more severe anemia [31, 32].

Other aspects of neurological function are coming under increased scrutiny in SCD. Autonomic nervous system dysfunction function is implicated by age-related impairment of heart rate recovery following maximal exercise testing in children with SCA [33]. Electroencephalograms and evoked potentials show abnormal neural networks in the brain, even without severe anemia, pain or silent infarcts [34]. These findings suggest subtle nervous system dysregulation that may affect the phenotype of SCD.

Altered brain connectivity detected by functional MRI is associated with increased frequency of hospitalization for pain, suggesting a model of central sensitization of pain in SCD [35]. In this model, multiple bouts of acute pain alter neuronal structure in the brain in some patients, triggering heightened perception of pain that promotes chronic pain. Data from sickle cell mice provide further detailed molecular and neurophysiological evidence for central sensitization occurring in the spinal cord, with increased pain signaling involving phosphorylation of mitogen-activated protein kinases (MAPKs), including c-Jun N-terminal kinase (JNK), p44/p42 extracellular signaling-regulated kinase (ERK), and p38 [36]. Inflammatory pathways also contribute to pain sensitivity in SCD mouse models, especially involving mast cells, and ligands of the nocioceptin and cannabinoid receptors appear to diminish neurogenic inflammation and pain in sickle cell mice [37, 38]. Clearly, great progress is being made to understand the mechanistic underpinnings of pain, the most important and universal clinical symptom of SCD, involving more sophisticated mechanisms than previously envisioned (Fig. 3).

Is Hydroxyurea a Panacea?

Coinciding with the twentieth anniversary of the pivotal study of hydroxyurea that led to its approval by the U.S. Food and Drug Administration for patients with sickle cell disease, new facets of hydroxyurea benefit were shown in 2015. No, it is not really a complete panacea for SCD, but published data continue to accumulate for expanding clinical use of the solitary FDA-approved SCD drug, both in terms of randomized clinical trial evidence and new preliminary findings (Table 2). In an SCD clinical research landmark study of children with SCD on chronic transfusion for abnormal transcranial Doppler velocity (TWiTCH), hydroxyurea has been shown to pose an acceptable alternative stroke prophylaxis, after a minimum of 1 year (mean 4 years) of chronic transfusion, accompanied in those with iron

overload by chelation therapy and phlebotomy [39]. These findings of this study were so strong that the interim analysis led to early study closure, and these results are expected to immediately change the standard of clinical care. In a related study hampered by inadequate accrual, post-hoc analyses supported a benefit of hydroxyurea therapy over observation in SCD children with borderline high TCD velocity, termed “conditional TCD velocity” (170-199 cm/sec). Although the latter results do not provide high-grade evidence, they do provide information that hydroxyurea does not pose unacceptable risks for conditional TCD patients, and it appears to reduce risk of progression [40]. Considering all of the other growing support for hydroxyurea therapy, this finding will likely lead to changes in clinical practice.

In the meantime, both basic science and clinical investigations continue to accumulate evidence of pleiotropic mechanisms and outcomes of benefit. Current dogma holds that the benefit of hydroxyurea takes months to accrue in clinical use. However, a single dose of hydroxyurea reduced vascular inflammation in sickle cell mice induced by experimental acute hypotonic hemolysis. This result may be due to the known NO donor properties of hydroxyurea, because they could be replicated by a well-known NO donor, DEANO [18]. Chronic hydroxyurea use was shown to reduce albuminuria in SCD, a complication that is epidemiologically linked to severity of hemolytic anemia, and to other hemolysis-linked complications such as elevated estimated pulmonary artery pressure [41]. Use of hydroxyurea was associated with normalization of effectors of angiogenesis and neovascularization that are prominent at baseline in SCD, including angiopoietin-1, basic fibroblast growth factor, vascular endothelial growth factor, vascular endothelial growth factor-D and placental growth factor, the latter of which has been linked also to elevated estimated pulmonary artery pressure [42]. Finally, use of hydroxyurea in SCD has been associated with apparently harmless cyclic rise and fall in platelet counts and absolute neutrophil counts, an apparent exaggeration of normal oscillatory hematopoiesis that should not be cause for alarm or reduction of dosing [43]. Previous reports have recognized this pattern only in patients with clonal myeloproliferative disorders, but now we can see that it can occur with hydroxyurea without intrinsic marrow disease.

Inflammation: Role of Dysregulated Innate Immunity in SCD

Recent observations are helping to characterize the mechanisms and impact of leukocytosis and inflammation in SCD (Fig. 2). Placenta growth factor, a VEGF family member overexpressed in SCD and now known to be stimulated by erythropoietin, heme iron and inflammatory cytokines, promotes to airway hyperreactivity in sickle cell mice via leukotrienes and IL-13 [44]. Thus, placenta growth factor appears to inflammatory pathways in the reactive airway disease frequently encountered in children with SCD. Turnover of heme iron in peripheral blood mononuclear cells (probably monocyte-macrophages) in SCD patients is associated with profoundly increased expression of the Toll-Like Receptor-4 (TLR4) - inflammasome system [45]. In particular, the transcript for TLR4, the initiator danger molecule receptor for the inflammasome pathway, was expressed at levels 200-fold that of healthy controls. This profound upregulation of the innate immune system in SCD was supported by a meta-analysis of gene expression studies [46]. If the TLR4 system is primed for ligand binding to activate inflammasome-mediated inflammation in SCD, what

could be the source of TLR4 ligand? A strong candidate for a source of lipopolysaccharide (a potent canonical ligand of TLR4) is normal gut bacteria. In my opinion the best article of 2015 in new sickle cell mechanistic research, investigators in Paul Frenette's laboratory demonstrated in sickle cell mice that antibiotic depletion of gut bacteria reduced vaso-occlusion observed by intravital microscopy, and improved splenomegaly and liver fibrosis, necrosis and inflammation [47]. These breakthrough data suggest that translocation of gut bacteria to the bloodstream may activate the primed TLR4 receptor, activating downstream inflammatory cytokines, promoting neutrophil and endothelial activation and adhesiveness, which much evidence suggests to be the initiating step to vaso-occlusion.

Adhesion of Circulating Blood Cells to Endothelium

The clinical importance of blood cell and endothelial adhesiveness is not completely proven, but investigational drug development provided important support to this mechanism in 2015. Rivipansel (originally known as GMI-1070) is an investigational small molecule blocker of E-selectin and P-selectin, important adhesion molecules that are highly expressed on endothelial cells and platelets in SCD and in inflammatory states (Fig. 2). Selectins provide the first point of contact on endothelium for activated flowing neutrophils to slow down, ultimately becoming immobilized via cell adhesion integrins. A phase 2 randomized controlled trial of rivipansel during vaso-occlusive pain crisis yielded a reduction in time to resolution of vaso-occlusive events and decreased opioid use, prompting initiation of a phase 3 trial [48]. An anti-platelet agent, prasugrel, was tested for its prophylaxis against occurrence of vaso-occlusive events, and showed only a nonsignificant favorable trend [49]. Although the failure of prasugrel to provide statistically significant protective effects is very disappointing, there were two bright spots. First of all, the protective trend supports cautious optimism that platelet activation is contributory to sickle cell vaso-occlusion and can be suppressed to some degree, providing hope that a more effective anti-adhesive approach might be successful in the future. Secondly, this clinical trial enrolled more patients than any other randomized controlled drug trial in the history of sickle cell disease, 341 patients from 51 sites in 13 countries. This accomplishment provides an important message to the pharmaceutical industry that large, informative trials can be conducted in SCD, the many willing investigators and study subjects disproving the pessimistic view that SCD patients will not participate in clinical trials in sufficient number.

Another anti-adhesive approach is the use of intravenous immunoglobulin G (IVIG), a product long in clinical use for other indications. In a phase 1 study in patients with SCD, single-dose IVIG was tolerable and stabilized neutrophil Mac-1 activation during sickle cell vaso-occlusive pain crisis [50]. Finally, a new anti-adhesive strategy was promising in sickle cell mice challenged with tumor necrosis factor alpha (TNF-α) or hypoxia/reoxygenation, using inhibitors of AKT2 in combination with hydroxyurea that reduced neutrophil adhesion, platelet-neutrophil aggregation, endothelial E-selectin and intercellular adhesion molecule 1, and improving survival [51]. The early evidence of improved outcomes in SCD mice and patients following treatment with investigational anti-adhesive therapy appears very promising, and supports the importance of this pathway in the pathophysiology of SCD.

Conclusion

Never in the history of sickle cell disease have so many potential new treatments seemed so imminently available. The pharmaceutical industry has teamed up with academia in unprecedented fashion in SCD drug development research. The future clearly holds more clinical trial data yet to emerge, although we must expect that there are always more negative than positive drug trials in any disease. Other publications in 2015 show the promise of new modalities to characterize and even cure SCD. An explosion of microfluidics technology has reached SCD research, and quantitative microfluidic microscopy methods were unveiled to study vaso-occlusion and retention of erythrocytes in the spleen in SCD [52, 53]. Innovative strategies were used in SCD mice to induce immune tolerance before birth, followed by curative postnatal non-myeloablative allogeneic hematopoietic stem cell transplant [54]. Finally, more progress was gained in gene therapy correction of the sickle cell disease mutation in human hematopoietic stem cells and progenitor cells [55]. The nearly 1200 publications on sickle cell disease in 2015 attest to a golden age in SCD, marked by renewed interest and accomplishments by investigators and pharmaceutical companies. They raise great optimism for progress in 2016 and beyond in the understanding and treatment of SCD.

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Key points

- Red blood abnormalities continue to underlie the complex pathophysiology of sickle cell disease.
- Vasculotoxic products of hemolysis contribute to dysregulated vasomotor function, inflammation, endothelial adhesiveness and clinical phenotype.
- Sensitization of the nervous system to pain stimuli underlies the pain experience in SCD.
- Clinical and experimental data are compelling for extending the use of hydroxyurea in SCD.
- A robust pipeline of investigational approaches for SCD appears more promising than ever before.

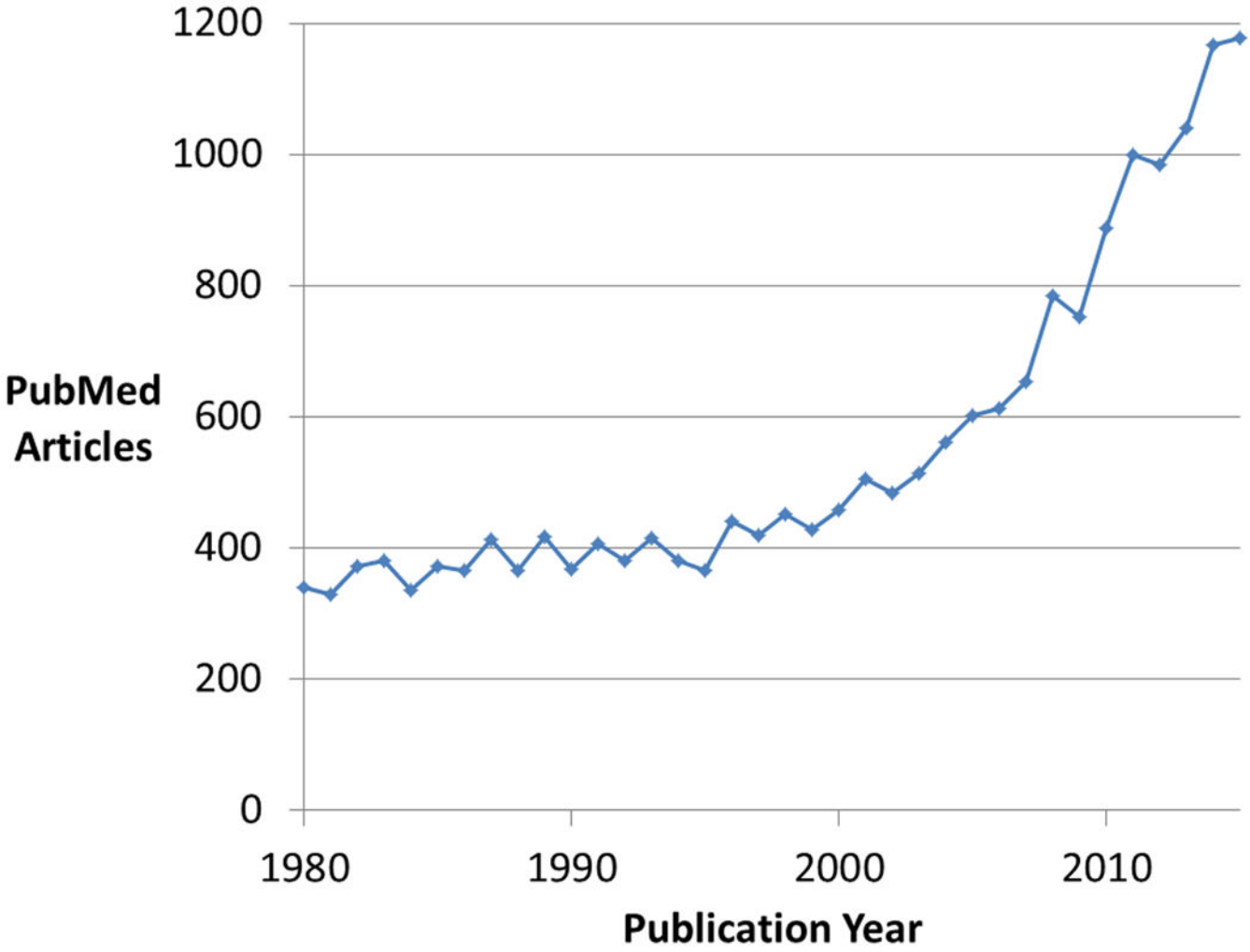

Figure 1. Publications each year involving sickle cell

This graph represents the annual publication rate of articles found on PubMed with the search term “sickle,” averaging 391 per year between 1980 and 2000. During the last 15 years, the publication number has increased by an average of 51 publications each year, rising from approximately 400 to nearly 1200 per year.

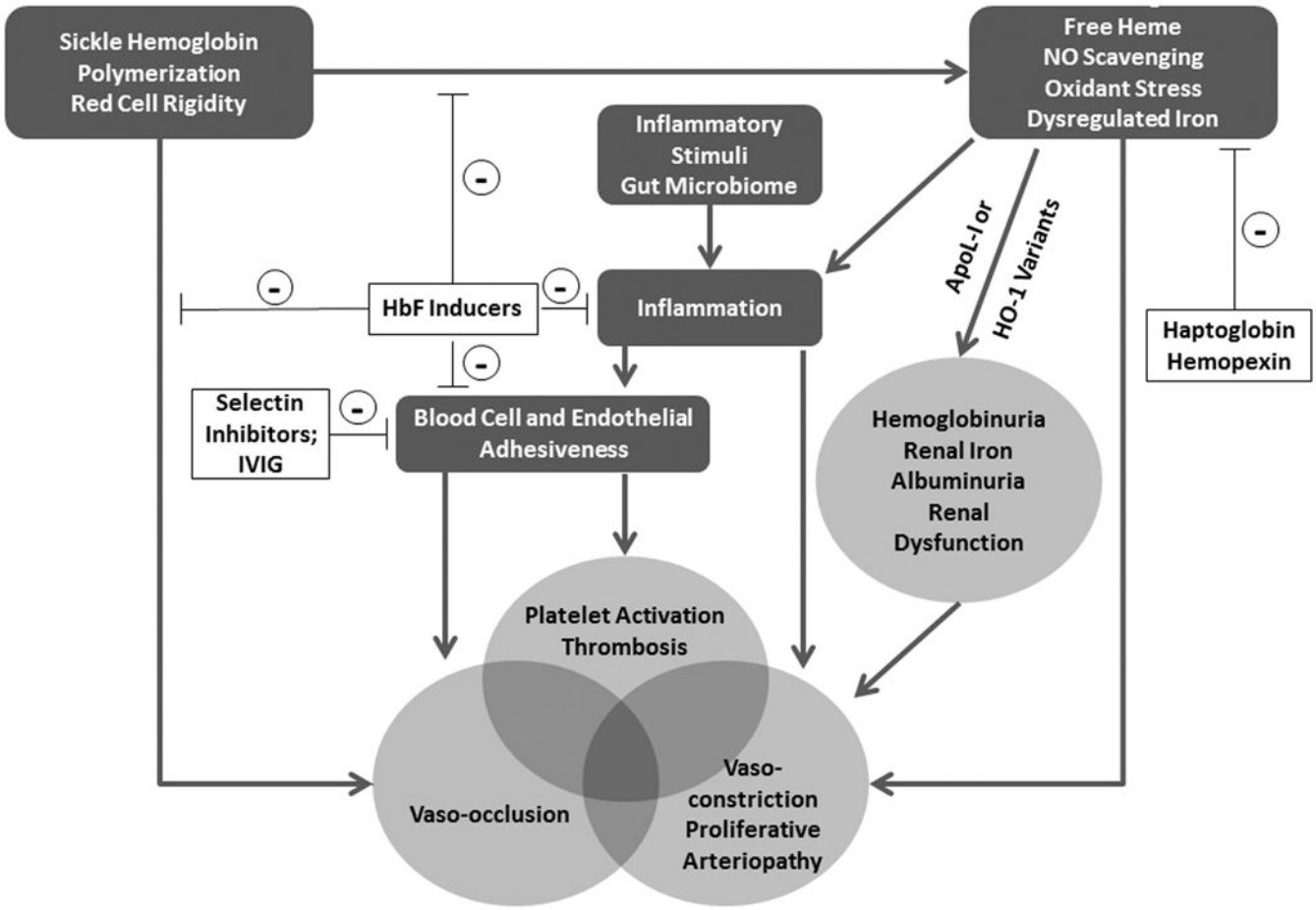

Figure 2. Key Insights from 2015 Involving Sickling, Thrombosis and Vasculopathy

Dark boxes include altered physiology in SCD, gray boxes include altered outcomes, and white boxes include current attempted therapeutic interventions being described in patients or mouse models. This figure summarizes highlights from new evidence published in 2015 and is not comprehensive. NO, nitric oxide; HbF, fetal hemoglobin; apoL-I, apolipoprotein L-I; HO-1, heme oxygenase-1; IVIG, intravenous immunoglobulin G.

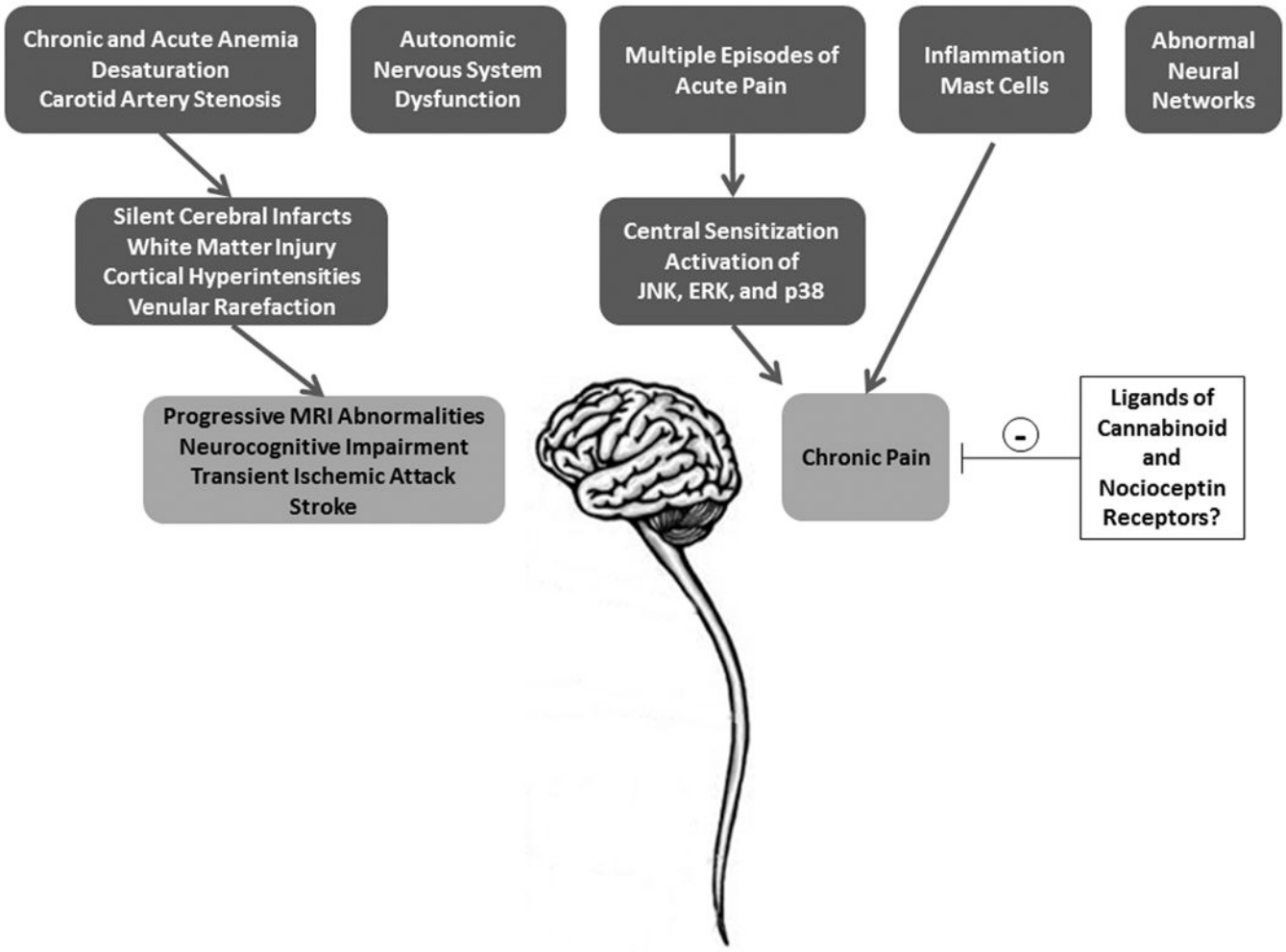

Figure 3. Key Insights from 2015 Involving the Brain and Pain

Dark boxes include altered physiology in SCD, gray boxes include altered outcomes, and white boxes include current attempted therapeutic interventions being described in patients or mouse models. This figure summarizes highlights from new evidence published in 2015 and is not comprehensive. MRI, magnetic resonance imaging; JNK, c-Jun N-terminal kinase; ERK, p44/p42 extracellular signaling-regulated kinase; p38, mitogen-activated protein kinase.

Table 1

Summary of Evidence from 2015 for Associations in Vasculopathy, Hemolytic Anemia and Nitric Oxide in Sickle Cell Disease

Findings from human and animal model studies.

Feature	Associated Finding
Right ventricular remodeling and impaired right ventricular function	• Impaired exercise tolerance and earlier mortality
Elevated estimated pulmonary artery systolic pressure	• Impaired exercise tolerance and earlier mortality (meta-analysis of 45 studies) • Systemic endothelial dysfunction
Free plasma hemoglobin	• Elevated estimated pulmonary artery systolic pressure • Systemic endothelial dysfunction • Vascular inflammation, normalized by nitric oxide donors
Plasma free heme or microvesicle heme	• Tissue factor-dependent activation of coagulation • Heme transfer to endothelium • Vaso-occlusion in sickle cell mice • Pro-inflammatory phenotype of macrophages in sickle cell mice, normalized by administration of hemopexin
Chronic renal injury with albuminuria	• Markers of intravascular hemolysis • Hemoglobinuria • Iron deposition in the kidney • Allelic variants in heme scavenging pathways
Extravasation of hemoglobin	• Impaired signaling by nitric oxide, normalized by administration of haptoglobin

Table 2
Documented and Putative Benefits of Hydroxyurea in SCD

Features	Randomized Controlled Trial	Other Clinical Trials	Mouse Models
Decreased acute pain, acute chest syndrome	✓		
Decreased transfusion requirements	✓		
Primary stroke prophylaxis after chronic transfusion	✓		
Primary stroke prophylaxis for conditional TCD velocity	✓		
Decreased vascular inflammation			✓
Improved albuminuria		✓	
Normalized circulating angiogenic factors		✓	
Improved estimated pulmonary artery pressure		✓	
Benign oscillations in platelet and leukocyte count		✓	