Minireview

Nitric oxide and sickle cell disease—Is there a painful connection?

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Impact statement

For the past two decades, researchers studying the pathophysiology of sickle cell disease (SCD) have hypothesized that the bioavailability of the endogenous vasodilator nitric oxide (NO) is decreased in SCD and that this NO deficit could contribute to SCD-related organ injury as well as acute pain episodes. However, multiple clinical trials aimed at increasing NO-production or release using NO-potentiating agents or NO itself yielded no significant alteration or improvement on the course of acute pain crises in SCD patients. This work provides a review of the contradicting results surrounding that hypothesis and proposes an alternative framework of thought regarding NO's role as a neurotransmitter possibly exacerbating SCD-pain.

Abstract

Sickle cell disease is the most common hemoglobinopathy and affects millions worldwide. The disease is associated with severe organ dysfunction, acute and chronic pain, and significantly decreased life expectancy. The large body of work demonstrating that hemolysis results in rapid consumption of the endogenous vasodilator nitric oxide, decreased nitric oxide production, and promotion of vaso-occlusion provides the basis for the hypothesis that nitric oxide bioavailability is reduced in sickle cell disease and that this deficit plays a role in sickle cell disease pain. Despite initial promising results, large clinical trials using strategies to increase nitric oxide bioavailability in sickle cell disease patients yielded no significant change in duration or frequency of acute pain crises. Further, recent investigations showed that sickle cell disease patients and mouse models have elevated baseline levels of blood nitrite, a reservoir for nitric oxide formation and a product of nitric oxide metabolism, regardless of pain phenotype. These conflicting results challenge the hypotheses that nitric oxide bioavailability is decreased and that it plays a significant role in the pathogenesis in sickle cell disease acute pain crises. Conversely, a large body of work demonstrates that nitric oxide, as a neurotransmitter, has a complex role in pain neurobiology, contributes to the development of central sensitization, and can mediate hyperalgesia in inflammatory and neuropathic pain. These results support an alternative hypothesis: one proposing that altered nitric oxide signaling may contribute to the development of neuropathic and/or inflammatory pain in sickle cell disease through its role as a neurotransmitter.

Keywords: Sickle cell disease, nitric oxide, nitrite, acute pain, chronic pain

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Introduction

Sickle cell disease (SCD) is the most common monogenic hematologic disorder and affects over 100,000 Americans and millions worldwide.[1,2] The disease is caused by a single point mutation in the gene encoding the β globin subunit, which results in the expression of a mutant sickle hemoglobin (HbS).[3,4] Upon red blood cell deoxygenation and/or dehydration, HbS polymerizes, which leads to sickling of red blood cells, ongoing chronic hemolysis, and recurrent episodes of vaso-occlusion.[3,5,6] These events result in chronic degenerative complications and as a result, SCD patients have a two- to three-decade reduction in life expectancy compared to the general population.[7] Patients with SCD not only face premature mortality, but also have a poor overall quality of life due to acute and chronic pain and varying degrees of multi-system organ damage.[2,8,9] In fact, SCD-related pain is a leading cause of morbidity, the main reason why SCD patients seek medical attention, and is associated with high health care costs. Data from 2018 show that acute pain episodes comprised a majority of the $2.98 billion total cost of care for SCD patients in the US.[10,11] Therefore, SCD pain is associated with significant morbidity and high health care costs.

Unfortunately, the pathobiology and triggers of SCD acute pain crises are incompletely understood. Further, while acute pain crises are the hallmark of the disease, many SCD patients can develop chronic pain also by incompletely understood mechanisms. For the last two decades, many experimental and clinical studies have focused

on the hypothesis that nitric oxide (NO) depletion and subsequent impaired vasodilation might play a role in the pathobiology of SCD complications including vaso-occlusion and acute pain. Indeed, among many functions, NO serves as a neurotransmitter and has been shown to be involved in the nociceptive process, in the development of central sensitization, and in mediating effects of analgesics including opioids.[12] While it is known that NO plays a complex and dual (pro- and anti-nociceptive) role in modulating nociceptive processing, it remains unknown whether NO availability is depleted in SCD and whether altered NO availability contributes to SCD-pain. In this short review, we discuss the complex relationship between NO and pain phenotypes in the context of SCD acute and chronic pain and posit that additional investigations are needed to understand whether that is indeed a connection between NO availability and SCD pain.

Sickle cell disease, a painfully complex disorder

While the molecular etiology of SCD has been known since Linus Pauling and colleagues published their study in *Science* over 70 years ago,[13] the mechanisms and triggers underlying SCD acute pain episodes, often referred to as vaso-occlusive crises (VOC), remain incompletely understood. A multiplicity of complex events is believed to take place and ultimately contribute to the development of vaso-occlusion and acute pain crises.[14–20] Some of those events include the sickling of red blood cells in a background of existing chronic inflammation and endothelial damage, which can lead to the activation of neutrophils, mast cells, macrophages, and platelets, the release of inflammatory cytokines and cell adhesion molecules from leukocytes and endothelial cells, and an increase in platelet aggregation.[3,21–23] This cascade of events thus illustrates the complexity of the underlying mechanisms of acute pain crises in SCD.

Given that few effective mechanism-based interventions are available, the current clinical approach to pain therapy for SCD acute pain crises is aimed at symptom control. High doses of opioid analgesics and fluids remain the mainstay of treatment during acute crises.[24] One of the many challenges clinicians face when treating SCD-pain is the remarkable variability of pain phenotypes among SCD patients. Researchers have shown that 1% of SCD patients experience more than six acute pain crises per year, while 39% of patients report few or no episodes of severe pain yearly.[25,26] Additionally, only 5% of SCD patients account for over 30% of all reported acute pain episodes requiring hospitalization.[25,26] While the underlying reasons for such variability in SCD-pain phenotypes are incompletely understood, factors such as sickle cell genotype, genetic modifiers, age, and disease severity appear to play a role.[25–27]

There is mounting evidence demonstrating that some SCD patients will develop chronic pain, which can be punctuated with acute pain crises. In addition to recurrent episodes of pain crises, complications of SCD, such as skin ulcers and avascular necrosis can be associated with acute and chronic pain.[28–30] This heterogeneity of pain phenotypes among SCD patients can certainly lead to therapeutic challenges and add complexity to the interpretation of results of clinical trials examining analgesic therapies. Typically, children with SCD are pain-free between acute pain crises and do not require chronic opioids.[31] In contrast, as patients get older, some will persistently have some degree of pain between crises.[31] In fact, publications reporting daily pain assessments of SCD patients show that more than 50% of patients experienced pain on more than half of the days evaluated.[26] Additionally, patient reported outcome studies using validated cross-sectional questionnaires also document that some SCD patients have pain with neuropathic characteristics, such as hypersensitivity or allodynia.[32,33] One study reported that almost 40% of the patients sampled used neuropathic pain descriptors to relate their pain experiences.[32] In 2017, the American Pain Society Pain Taxonomy initiative developed an outline for the diagnosis of chronic SCD pain syndromes.[34] The SCD experts in that working group proposed the existence of three common subtypes of chronic pain in SCD patients: chronic pain not resulting from SCD complications, chronic pain derived from a complication of SCD, and chronic pain with mixed presentation.[34] In turn, recently, the American Society of Hematology issued a set of specific guidelines for the management of acute and chronic pain in SCD patients.[28] Further, recognizing that SCD patients have such high pain burden, scientific societies as well as regulatory and funding agencies have dedicated great effort and resources to improve our understanding of the underlying mechanisms of SCD pain and to develop effective mechanism-based non-opioid pain therapies.

The NO deficiency hypothesis in SCD

During the past 20 years, a number of preclinical [35–37] and clinical studies [35,38–44] have centered on the hypothesis that decreased NO availability and signaling play a central role in the pathobiology of SCD complications including vaso-occlusion and pain. Several molecular events could certainly increase NO scavenging and decrease NO production, thereby leading to decreased NO bioavailability (Figure 1) in SCD. Sickling and hemolysis of red blood cells caused by HbS polymerization result in the release of cell free hemoglobin that rapidly and potently scavenges NO from plasma,[14,45] thereby increasing NO consumption. Hemolysis also results in the release of arginase, an enzyme that degrades the substrate for NO synthesis, L-arginine, effectively decreasing NO production by NO synthase (NOS) enzymes. In fact, plasma arginine levels have been shown to be reduced in SCD patients compared to controls.[46] Further, arginine deficiency resulting from increased arginase activity has been linked to decoupling of NOS enzymes and the production of superoxide that then reacts with NO to form peroxynitrite, which further reduces NO bioavailability.[46,47] Vascular inflammation and superoxide production in SCD potentially result in a deficiency of the essential NOS cofactor, tetrahydrobiopterin, which has been implicated in the uncoupling of NOS enzymes, rendering them unable to produce NO.[48–50]

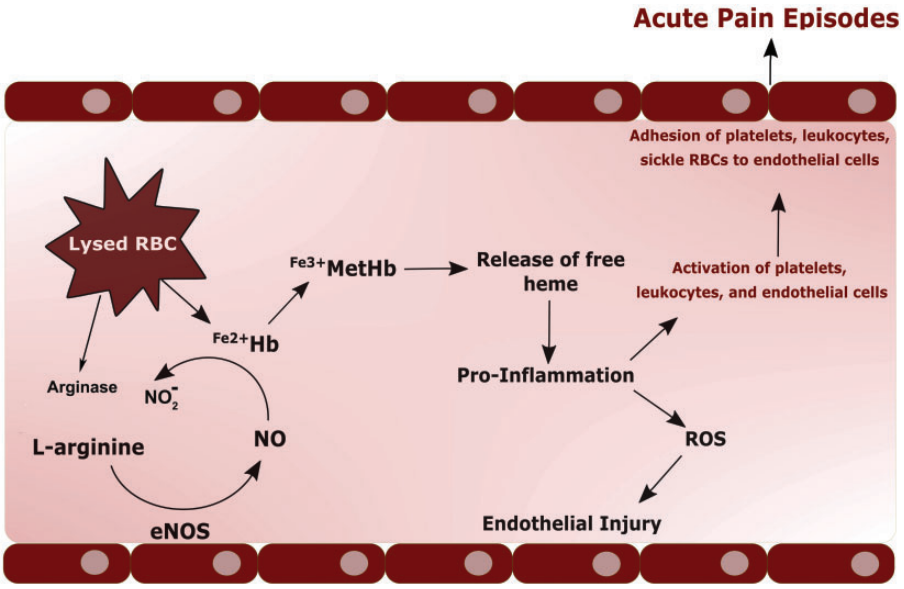

Figure 1. The NO-deficiency hypothesis. Hemolysis of red blood cells (RBCs) in sickle cell patients results in the release of free ferrous hemoglobin (Fe2+Hb) that then rapidly reacts with nitric oxide (NO) to form nitrite (NO_2) and methemoglobin (Fe3+MetHb). This results in the release of free heme, which promotes inflammation, oxidative stress with reactive oxygen species (ROS) production, and endothelial injury. The release of free heme additionally activates platelets, leukocytes, and endothelial cells, leading to their adhesion to the endothelium. Hemolysis also results in the release of arginase, an enzyme that degrades the substrate for NO synthesis, L-arginine, effectively decreasing NO production by NO synthase (NOS) enzymes. This complex cascade of events is believed to contribute to vaso-occlusion, downstream ischemia, and possibly acute pain crises.

Moreover, SCD patients have been shown to have elevations of asymmetric dimethylarginine (ADMA), the arginine metabolite and endogenous NOS inhibitor, implying increased eNOS inhibition and decreased NO production.[51] Therefore, the "NO deficiency hypothesis" postulates that various events triggered by hemolysis result in decreased NO bioavailability in SCD. In turn, decreased NO bioavailability would lead to vasoconstriction, endothelial injury, reactive oxygen species generation, platelet activation, leukocyte adhesion, vaso-occlusion, and downstream ischemia, possibly contributing to the development of acute pain crises.

These findings and concepts have formed the basis for several clinical trials of strategies aimed at increasing NO bioavailability in SCD patients to treat a number of SCD complications including acute pain crises and other vascular complications such as chronic kidney disease, stroke, and pulmonary hypertension.[40,41,52] While the theory behind this hypothesis has been reviewed in great detail,[53,54] investigations into the extent that reduced NO bioavailability contributes to the pathophysiology of SCD acute pain episodes have produced conflicting results.[35,38,55,56]

The challenges of measuring nitric oxide levels in biological matrices—The proof is in the measurement

Studies of NO availability and its signaling are fraught with challenges related to its measurement given NO's very short half-life (100 ms half-life average) and the complexity of its metabolism.[57,58] Nitric oxide rapidly reacts with hemoglobin, is metabolized into nitrite and nitrate,[59,60] and can react with lipids, proteins, and thiols to form peroxynitrite, N-nitrosamines (RNNOs), and S-nitrosothiols (RSNOs).[61,62] Several of these NO-generated compounds actually have physiological functions and can regenerate NO.[62,63] Therefore, the challenges associated with the measurement of NO are not to be underestimated and need to be considered when interpreting studies of its bioavailability.

The NO metabolites, nitrite, and nitrate, are commonly measured in combination and referred to as NO_x, which is a surrogate measure of NO levels. However, NO_x measurements tend to reflect mostly nitrate levels, which are well known to vary greatly with human diets. The other NO metabolite, nitrite, serves as a reservoir pool for NO production and as a signaling molecule itself.[64,65] A great deal of work has shown that nitrite can re-generate NO by NOS-independent pathways, especially during hypoxic and ischemic conditions, and as such, nitrite can mediate vasodilation and cytoprotection in the setting of ischemia/reperfusion.[66–72] Therefore, specific measurement of nitrite has been regarded as an indirect measurement of NO availability.

One caveat when measuring nitrite levels is the fact that nitrite is commonly found in collection vials, laboratory glassware, and reagents, generating concern for sample contamination during collection, processing, and nitrite measurement.[73] Protocols for blood collection to avoid sample nitrite contamination have been developed and used to examine nitrite levels in blood compartments and biological matrices.[73,74] With such methods, the measurement of nitrite is an acceptable surrogate measure of NO. In addition to NO metabolites, such as nitrite and nitrate, secondary messengers such as cyclic guanosine monophosphate (cGMP) have been cited as potential indicators of downstream NO signaling and NO levels.

Altered NO availability in SCD: What is the evidence?

Apart from the characterization of a cascade of events that could lead to increased consumption and decreased production of NO, studies conclusively demonstrating decreased NO availability in SCD are lacking. In support of decreased NO bioavailability, one study showed that SCD patients with higher plasma heme concentrations, and therefore increased NO consumption, had lower forearm blood flow measurements, suggesting that increased NO scavenging by heme leads to decreased NO bioavailability and less vasodilation in SCD.[14] Researchers also investigated lactate dehydrogenase (LDH) as a biomarker of intravascular hemolysis and NO consumption.[75,76] In one study, researchers showed that patients with high LDH had more hemolysis, higher plasma NO consumption, and diminished vasodilatory responses to nitroprusside, a NO donor.[76] Further, those patients were found to be at increased risk of developing pulmonary hypertension, cutaneous leg ulcers, and priapism, and of having early mortality.[76] While the study was not powered to establish a relationship between high LDH and incidence of strokes or other disease complications, the authors concluded that increased hemolysis was associated with decreased NO availability and vascular complications in SCD patients.[76] Together, these studies provided indirect evidence that NO availability might be decreased in SCD.

Researchers have shown that SCD patients display diminished vasodilatory responses to NO donors and these finding led to the hypothesis of a "nitric oxide resistance state" in SCD patients.[77] However, studies investigating the vasodilatory responses of SCD patients to NO-dependent stimuli have produced conflicting results.[42–44] One study showed that forearm blood flow was higher in SCD patients than in healthy subjects in response to the eNOS dependent vasodilator acetylcholine,[43] thus suggesting an increased synthesis or release of NO in response to pharmacologic stimuli in these patients.[42,43] Interestingly, in that same study there was no significant difference in forearm blood flow changes upon administration of NG-Methyl-L-arginine (L-NMMA), a nonspecific NOS inhibitor, comparing SCD patients and healthy subjects, suggesting that both groups had similar baseline NO levels.[43] Moreover, administration of the NO donor, sodium nitroprusside, produced similar vasodilatory responses in SCD patients and healthy subjects, indicating normal NO-mediated vasodilation in SCD patients.[43] Together, these findings suggest normal basal production of NO and upregulated acetylcholine-dependent NO production, which is not consistent with impaired vessel diameter adjustments in SCD. These results are in agreement with other blood flow studies demonstrating increased vasodilatory response to NO donors or pharmacologic stimuli in SCD patients or mice compared to controls.[42,78] Together, these results dispute the hypothesis of decreased NO bioavailability and/or nitric oxide resistance in SCD.

Also contradicting the decreased NO availability hypothesis, there are multiple animal and human studies suggesting that nitrite and NO bioavailability are actually elevated, rather than reduced in SCD during steady state (baseline) conditions. For example, despite methodological limitations mentioned previously, researchers measuring plasma NO_x (nitrite and nitrate combined) concentrations in SCD patients and controls showed that these NO metabolites were elevated in SCD patients at steady state.[79] This was corroborated by a later study showing an eight-fold increase of plasma nitrite levels in SCD patients compared to control subjects during baseline conditions.[55] In addition to elevations of plasma nitrite, expression of endothelial NOS was shown to be increased in cremaster muscle and kidneys of transgenic SCD mice.[80,81] These increases in endothelial NOS expression were coupled with lower mean arterial pressure [80] and higher urinary nitrite measurements,[81] which were altered with NOS substrates and reversed with NOS inhibitors.[80,81] Others have examined the secondary messenger in the NO signaling pathway, cGMP, at baseline and showed that vascular cGMP is elevated in aortic rings of SCD mice.[37] The results of these studies were further supported by our recent study showing elevated nitrite and cGMP levels in blood compartments of sickle cell patients and mice when compared with respective controls.[35] Together, these findings further contradict the hypothesis that NO availability is decreased in SCD.

For proponents of the decreased NO availability hypothesis, the reported elevations of plasma NO metabolites and secondary messengers are regarded as paradoxical in the setting of endothelial dysfunction and hemolysis, which is characteristic of SCD[22]. While a molecular mechanism explaining such elevations has not been identified, a possible alternative explanation may consider that the elevation of nitrite levels and eNOS expression in SCD results from a compensatory response to chronic inflammation and vasoconstriction that is seen in SCD. We have recently shown that the accumulation of nitrite in blood of SCD patients and mice results in part from slowed nitrite metabolism caused by decreased hemoglobin concentrations observed in SCD patients and mice.[35] Although it is possible for cGMP and nitrite levels to be upregulated by NO-independent mechanisms, [82,83] the fact that nitrite, cGMP, and NOS expression levels are not decreased at steady state in SCD suggests that NO bioavailability is not decreased and might actually be increased in SCD patients.

NO's role (or lack thereof) in SCD acute pain

The extent to which reduced NO bioavailability contributes to the pathophysiology of SCD acute pain episodes has been examined in translational and clinical studies.[35,38,55,56] Studies demonstrating reductions of NO_x during acute pain episodes supported the notion that reduced NO bioavailability played a role in exacerbating pain episodes. One study measuring plasma NO_x in SCD patients at steady state and during acute pain crises showed a significant reduction in NO metabolites during acute pain episodes.[84] These results are corroborated by multiple other studies finding decreased NO_x during acute pain episodes compared to steady state.[85–87]

When we investigated the relationship between pain burden and blood compartment nitrite levels in SCD subjects, we found that subjects with low (defined as ≤ 2 pain related hospitalizations per year) and high pain burden (defined as ≥ 3 pain related hospitalizations per year) have similarly elevated nitrite levels.[35] Those findings thus suggest a lack of correlation between steady state nitrite levels, NO bioavailability, and yearly number of pain crises in SCD patients.[35] Another study measured serial serum NO_x levels and pain scores of SCD patients receiving emergency department analgesic intervention for VOC and showed no significant correlation between changes in NO_x levels and changes in pain levels during the overall treatment period. [38] Taken together, these findings suggest that nitrite/nitrate levels are unrelated to the frequency, severity, or duration of pain episodes in SCD patients or animal models.[38]

While in one study, researchers proposed that increased hemolysis would promote vasoconstriction and increase the frequency of acute pain crises,[76] the degree of hemolysis based on LDH levels does not correlate with the frequency of acute pain crises in a larger cohort of SCD patients.[88] In fact, a sub-group of those patients with chronically increased LDH exhibited fewer acute pain crises than patients with lower LDH values, suggesting that more hemolysis would actually be associated with fewer acute pain episodes.[88] Additionally, as pointed out by others,[89] the observed levels of plasma hemoglobin in SCD are 10-fold lower than those levels in paroxysmal nocturnal hemoglobinuria, another hemolytic disorder, which is not associated with acute pain episodes.[89] Combined, these results further challenge the hypothesis that an increase in NO scavenging by cell-free hemoglobin causes increased frequency of pain crises in SCD.

Increasing NO bioavailability failed to ameliorate acute pain in SCD patients

For years, hydroxyurea had been the only FDA-approved disease-modifying treatment for SCD and the mechanism of its salutary effects has been predominantly attributed to its effect on fetal hemoglobin synthesis. However, researchers have shown that hydroxyurea directly generates NO in erythroid cells [90] and that the hydroxyurea-associated induction of fetal hemoglobin is mediated by NO-dependent activation of soluble guanylate cyclase.[91] Additionally, others have shown that in models of tumor necrosis factor-α-induced acute vaso-occlusion in SCD mice, hydroxyurea improves leukocyte rolling and decreases leukocyte adhesion and red blood cell/leukocyte interactions, which were coupled with beneficial effects on vaso-occlusive mechanisms and survival.[92] That same group also showed that acutely, hydroxyurea ameliorates the rapid inflammatory response observed in models of acute hemolysis.[93] Importantly, in those two studies, these acute beneficial effects of hydroxyurea were not associated with increases in fetal hemoglobin and were mediated by NO donation and increased NO/cGMP signaling.[92,93] Together these reports suggest that in addition to increasing fetal hemoglobin, hydroxyurea might have acute salutary effects, which result from increases in NO/cGMP signaling.

These findings together with the notion that NO availability could be decreased in SCD formed the basis for clinical trials of therapeutic strategies aiming at increasing NO delivery/production (NO donors, arginine salt, increasing NOS activity) or NO signaling (phosphodiesterase inhibitors) in SCD patients. Unfortunately, the trials using strategies to increase NO availability conducted to date have failed to show benefits on the frequency and course of acute pain episodes, thus calling into question the decreased NO availability hypothesis and its role in modulating acute pain in SCD patients.[39–41,52] A multicenter clinical trial of sildenafil, a phosphodiesterase inhibitor, which inhibits cGMP degradation and increases NO signaling, was discontinued prematurely due to increased pain crises frequency among patients on sildenafil.[39,52] Furthermore, a multi-institutional controlled trial of inhaled NO revealed no therapeutic benefit to adults with acute pain crises as length of hospital stay, visual analog pain scores, cumulative opioid use, and rate of acute chest syndrome remained unchanged compared with placebo.[40] Notably, oral arginine, a substrate for NOS, yielded a significant decrease in total analgesic use but no change in total length of hospital stay in pediatric SCD patients.[41] Together, these results contradict the idea that increasing NO availability benefits SCD patients during acute pain crises.

In SCD mice, an attempt at chronic nitrite supplementation was associated with elevations in plasma nitrite, increases in cGMP,[35] and a paradoxical decrease in muscle nitrite.[56] These decreases in muscle nitrite levels were associated with an improvement in grip force, suggesting an improvement in muscle function and in muscle hyperalgesia. In contrast, elevations in plasma nitrite and cGMP levels with nitrite supplementation yielded no changes in sensory fiber sensitization measured by thermal and electrical stimulation.[35] Given these results from preclinical and human trials, one must consider that further increases in NO availability might not be beneficial and, in some instances, may be detrimental. One must also consider the possibility that the effect of given treatments on frequency and hospital course of acute pain crises may not inform or predict the effects of those treatments on other complications of SCD. Nevertheless, while strategies to increase NO donation/nitrite supplementation may not be suitable approaches for SCD, it is worth noting that other phosphodiesterase inhibitors (phosphodiesterase-9) and cGMP amplifying agents are currently under various stages of investigation for the treatment of vascular SCD complications.[94–96] Whether these agents will have a role in acute pain crises or chronic pain in SCD is yet unknown.

The role of NO signaling in pain—A balancing act

The role of NO signaling in the neurobiology of pain is complex in that it can have both, anti-and pro-nociceptive effects. For example, researchers have shown that activation of NO/cGMP signaling pathways by peripheral

administration of NO donors yields analgesia in models of peripheral inflammatory pain and that NOS and cGMP inhibitors block these analgesic effects.[97,98] Also supporting the anti-nociceptive effects of NO signaling are a number of preclinical investigations indicating that opioid-induced peripheral analgesia in inflammatory pain is dependent on the activation of NO/cGMP signaling in peripheral sensory nerves.[99] Further, reports suggest NO may transiently have anti-nociceptive properties within the spinal cord, depending on the type of neurons activated.[100] For example, intrathecal administration of L-arginine increased mechanical tail withdrawal thresholds in rats.[101] Together these studies support the notion that activation of NO/cGMP signaling can be beneficial in inflammatory pain by preventing and/or mitigating excitability of peripheral sensory neurons.

Conversely, a large body of literature implicates NO and its signaling in pro-nociceptive effects.[102,103] Animal studies have shown that inhibition of NO or cGMP production can reduce inflammatory and neuropathic pain.[102,104,105] For example, two studies showed that inhibition of NO synthesis by the non-specific NOS inhibitor L-NAME reduced thermal and mechanical hyperalgesia induced by inflammation.[106,107] Other studies have reported a reduction in inflammatory and neuropathic pain markers and associated nocifensive behaviors upon selective neuronal NOS (nNOS) and inducible NOS (iNOS) inhibition.[108–111] Additionally, administration of NO and cGMP activators have been shown in multiple studies to increase hypersensitivity of sensory neurons in pain models.[112–120] One proposed mechanism of its pro-nociceptive effects is that NO accentuates the dysregulated spreading potentiation of nociceptive signals in the spinal cord, which leads to neuropathic pain in hindpaw ischemia models.[112] This effect was effectively blocked by spinal application of L-NAME and is absent in neuronal NOS knockout mice.[112]

Another study investigating inflammatory pain in mice deficient in NOS isoforms showed significant reductions in thermal hyperalgesia in nNOS, eNOS, and iNOS knock out mice.[104] Interestingly, nNOS deficient mice had little thermal hyperalgesia and displayed no mechanical allodynia with inflammatory pain.[104] We have additionally shown that genetic deficiency of the three NOS isoforms nNOS, iNOS, and endothelial NOS (eNOS) differentially alters baseline nocifensive behavior.[121] Neuronal NOS-deficient animals had evidence of increased tolerance to electrical stimulation of myelinated (Aβ and Aδ) and non-myelinated (C) sensory nerve fibers, suggesting that inhibition of nNOS and subsequent decreases in neuronal NO attenuates sensory fiber hyperalgesia. Inducible NOS-deficient animals also had increased tolerance only to C-fiber stimulation, [121] with another study further implicating iNOS in inflammatory pain processing.[122] However, investigations of eNOS deficiency have produced conflicting results. Our study found that eNOS-deficient animals had decreased tolerance to Aβ and Aδ sensory fiber stimulation, [121] while other studies found no evidence to support that eNOS is involved in pain processing.[110] Together, these investigations indicate that alterations in NO production can significantly alter the phenotype of inflammatory and neuropathic pain models. While it is clear that NO may exert pro- and anti-nociceptive roles (Table 1), it remains unclear what exactly determines whether NO and cGMP signaling will serve nociceptive or anti-nociceptive functions in neuropathic or inflammatory pain.

Beyond acute pain: Implications for chronic pain syndromes in SCD

There are multiple lines of evidence supporting the presence of inflammatory and neuropathic components in SCD pain. Preclinical studies in SCD mice suggest that in SCD there can be dysregulation of sensory neuronal signaling within the peripheral and central nervous systems.[123–127] SCD mice display evidence of peripheral neuropathy manifested by thin epidermal skin layers and decreased innervation.[128] There are also extensive reports of hypersensitivity to mechanical and thermal stimuli in SCD mouse models, further supporting the existence of abnormalities of the peripheral nervous system and sensitization of sensory nerve fibers.[123,127,128] We have shown that SCD mice have sensitization of both myelinated (Aβ and Aδ sensory fibers) and non-myelinated (C) sensory fibers thus suggesting a neuropathic component to the nociception phenotype in the model.[127] Furthermore, SCD mice exhibit increased expression of calcitonin gene related peptide (CGRP) and substance P in skin layers, and exhibit increased expression of TLR-4 and inflammatory cytokines such as IL-6 in spinal cords, all of which are upregulated in neuropathic and inflammatory pain conditions.[128] Together these preclinical studies suggest that central sensitization

Table 1. Dual effect of nitric oxide (NO) and cyclic guanosine-3′,5′-monophosphate (cGMP) signaling in nociception.

Anti-nociceptive effects of nitric oxide signaling	Pro-nociceptive Effects of nitric oxide signaling
Increased NO/cGMP signaling yields anti-nociceptive responses to prostaglandin-E2 induced hyperalgesia[119]	NO contributes to development and maintenance of central sensitization[115]
Peripheral opioid analgesia is dependent on NO/cGMP signaling[96]	NO/cGMP signaling mediates glutamate induced hyperalgesia[110]
L-arginine increases mechanical thresholds in rats[98]	Neuronal NOS knockout mice show 50% reduction in thermal hyperalgesia and abolished mechanical hyperalgesia in models of inflammatory pain[101] NOS inhibition attenuates nerve-injury induced mechanical hyperalgesia[102] and reduce thermal and mechanical hyperalgesia in inflammatory pain models[97,103,104,112,127]

NOS: nitric oxide synthase.

and neuropathic processes could contribute to the development of chronic pain in SCD.[33,34]

Given the known role of NO/cGMP signaling in nociception, in the context of SCD, one must consider the possibility that elevations of NO metabolites and cGMP could contribute to inflammatory and neuropathic pain syndromes in SCD patients.[35,55,56,79] As discussed here, SCD patients (and mice) have elevated blood nitrite and cGMP levels in plasma and red cells at baseline, and while those elevated levels do not correlate with the frequency of acute pain episodes, they are associated with sensory fiber sensitization. Given these findings and the evidence suggesting that altered NO bioavailability can contribute to neuropathic and inflammatory pain syndromes, a hypothesis that increased NO availability could contribute to the development of chronic pain syndromes in SCD is reasonable and worthy of testing. Further, research to determine whether there are alterations of NO and cGMP signaling at the level of sensory neurons and central nervous system and to determine the triggers and mechanisms underlying acute pain crises and chronic pain syndromes in SCD patients are certainly needed. In this regard, we propose a shift in the framework of thought in order to understand the direct pathophysiological consequences of accumulated nitrite and cGMP signaling in SCD to determine whether there is indeed a painful connection.

AUTHORS' CONTRIBUTIONS

All authors participated in the writing and review of the manuscript.

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