Pain and sickle cell disease

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Purpose of review
Pain is a major comorbidity of sickle cell disease (SCD). Opioids are the mainstay for pain treatment but remain suboptimal. We discuss mechanism-based treatable targets devoid of opioids to prevent and/or treat SCD pain.

Recent findings
Understanding the pathogenesis of pain is critical to develop targeted therapies. Nevertheless, acute and chronic pain can have independent and/or overlapping mechanisms. The origin of pain involves neurovascular and neuroimmune interactions from the periphery and/or central nervous system. Immunomodulatory components of acute and/or chronic sickle pain for targeting/preventing pain genesis include mast cell and microglial activation, neurogenic inflammation, and leukocyte-derived elastase. Vascular modulators include hypoxia/reperfusion injury, oxidative stress, hemolysis, and adhesion molecules. However, existent pain requires analgesics devoid of an inadvertent effect on sickle pathobiology. Recent analgesic targets include cannabinoid and nociceptin receptors and serotonergic spinothalamic pathway. Complementary approaches (e.g., acupuncture, hypnosis, perception-based therapies) have shown analgesic potential. Owing to heterogeneity in pain development, it remains challenging to combat SCD pain with any one therapy.

Summary
SCD pain involves neuroimmune and neurovascular interactions. Such interactions have pronociceptive impacts and impart therapy resistance. Elucidating molecular and cellular entities affecting neuronal interactions in sickle microenvironment may prevent SCD pain and/or provide improved analgesic approaches.

Keywords
elastase, mast cells, neuroimmune interaction, sickle pain, vasoocclusion

INTRODUCTION

Sickle cell disease (SCD) is the most common monogenic vascular disorder worldwide and the affected population is projected to increase in future decades [1▪▪,2▪,3]. SCD results from a single point mutation in the β-hemoglobin gene, leading to the characteristic sickle shape of red blood cells (RBCs) [4,5]. Pain is a major comorbidity of SCD [6▪,7,8].

Opioids are the mainstay of therapy for both acute and chronic pain in SCD but remain a suboptimal approach because of liabilities, including fear of addiction [6▪,9–12]. Moreover, the current national opioid crisis may negatively impact the treatment of pain in SCD with opioids [11]. Therefore, an emergent need is to develop targeted therapies devoid of opioid use. However, the complex neurobiology of sickle pain is not well understood but involves neuropathic, inflammatory, and nociceptive causes. Moreover, acute and chronic pain may coexist with overlapping pathobiologic features. Pain research is targeted toward treating existant pain. Nevertheless, preventing pain from being evoked at its source is critical to improve the quality of life in SCD. Humanized mouse models expressing exclusively human sickle hemoglobin such as Berkley transgenic mice have provided critical insights into the mechanisms underlying pain in SCD [13▪▪,14]. Here, we discuss the promise and challenges of mechanism-based treatable targets to prevent and/or treat pain in SCD.

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KEY POINTS

- Pain is a major comorbidity of SCD requiring opioid analgesia.
- An unmet need is to develop targeted therapies to prevent pain from being evoked.
- Neuroimmune and neurovascular mechanisms of acute and chronic pain identified include neurogenic inflammation, and activation of mast cells, leukocytes, and adhesion molecules.
- Nonpharmacologic integrative approaches, including nutrition, mindfulness, hypnosis, and lifestyle changes, may moderate pain.
- Objective, mechanism-based biomarkers for targeting appropriate therapies to optimize analgesic outcomes for individuals are warranted.

PATHOBIOLOGY OF ACUTE SICKLE PAIN

Acute pain is associated with unpredictable, intractable, and episodic vasoocclusive crises (VOC) leading to hospitalization and poor quality of life [15,16]. VOC is a complex vascular phenomenon involving multiple cellular and molecular cascades orchestrating blockage of blood flow in microcapillaries leading to ischemia/reperfusion injury and intractable pain [1▪▪,17]. Repeated cycles of sickling–unsickling lead to damaged RBCs making them more adhesive because of activation of the Lutheran adhesion glycoprotein/basal cell adhesion molecule, $\alpha_4\beta_1$ receptors, and intercellular adhesion molecule (ICAM)-4 [18–20]. Moreover, the highly hemolytic and resultant oxidative sickle microenvironment, in addition to interactions between adhesive RBCs and the endothelium, enables endothelial activation of adhesion molecules such as platelet selectin (P-selectin), endothelial selectin (E-selectin), vascular cell adhesion molecule 1, and ICAM-1 via nuclear factor kappa-light-chain-enhancer of activated B cells-mediated mechanisms [21,22]. Persistent hemolysis contributes to endothelial activation via oxidative damage, neutrophil activation, and the formation of neutrophil extracellular traps (NETs) [23]. Adhesive aggregates of RBCs, leukocytes, and platelets block vascular blood flow leading to VOC (Fig. 1).

MECHANISM-BASED TREATABLE TARGETS FOR ACUTE PAIN

RBC–endothelium and platelet–leukocyte interactions, which promote VOC, are known to be orchestrated by adhesion molecules including P-selectin [21,22,24,25]. Crizanlizumab, an anti-P-selectin antibody, was shown to reduce VOC events in patients with high frequency of VOCs (5–10) in the previous year, in a Phase II clinical trial [26]. Additionally, rivipansel, a pan-selectin inhibitor for both E-selectin and P-selectin, is in clinical trial for resolution of VOC. Initial results indicate that patients in crises treated with rivipansel recovered much faster than patients on placebo [27]. L-Arginine treatment to increase nitric oxide-assisted vasodilation during VOC showed improvement but did not show statistical significance in the pain outcomes [28]. In 2017, the L-glutamine supplement Endari was approved by the US FDA as a new drug for reducing acute VOCs. In phase III clinical trials, the patient group receiving Endari developed less pain crises (3.0) and fewer hospitalizations (2.0) compared with placebo group (4.0 and 3.0, respectively) [29]. However, side-effects of Endari include fatigue, noncardiac chest pain, and musculoskeletal pain. The mechanism of action of Endari has not yet been elucidated, in SCD. Glutamine is metabolized into glutamate involving the release of nicotinamide adenine dinucleotide hydrogen, which in turn is converted to NADPH. Endari may, therefore, increase NADPH in RBCs, which decreases oxidative stress [17]. As a consequence, reduced sickle hemoglobin polymerization may occur because of a reduction in intra-RBC heme release [30]. In contrast, glutamate may contribute to the hyperexcitability of the nociceptors, because glutamate is a known excitatory neurotransmitter and mediator of pain [31].

GENESIS OF ACUTE PAIN AND TRANSITION TO CHRONIC PAIN

Depletion of nitric oxide, reduced fetal hemoglobin, hemolysis, upregulation of soluble and cell surface adhesion molecules, modulation of coagulation pathways, inflammation, oxidative stress, and hypoxia/reperfusion injury contribute to impaired vascular health is recognized. How this vascular disease leads to acute pain remains an enigma. Additionally, although sickle cell trait (SCT; inheritance of only one copy of the mutated β-hemoglobin gene) was once regarded as benign with respect to sickle pain, SCT mice were shown to have increased mechanical hypersensitivity following hypoxia/reoxygenation [32].

The noxious environment created by vascular inflammation, excess cell-free heme, and release of neuropeptides from activated inflammatory cells stimulate nociceptors on peripheral nerve terminals (Fig. 1). Additionally, tissue-resident granulocytes such as activated mast cells may lead to direct nerve injury via their extensions and traps [33], as detailed

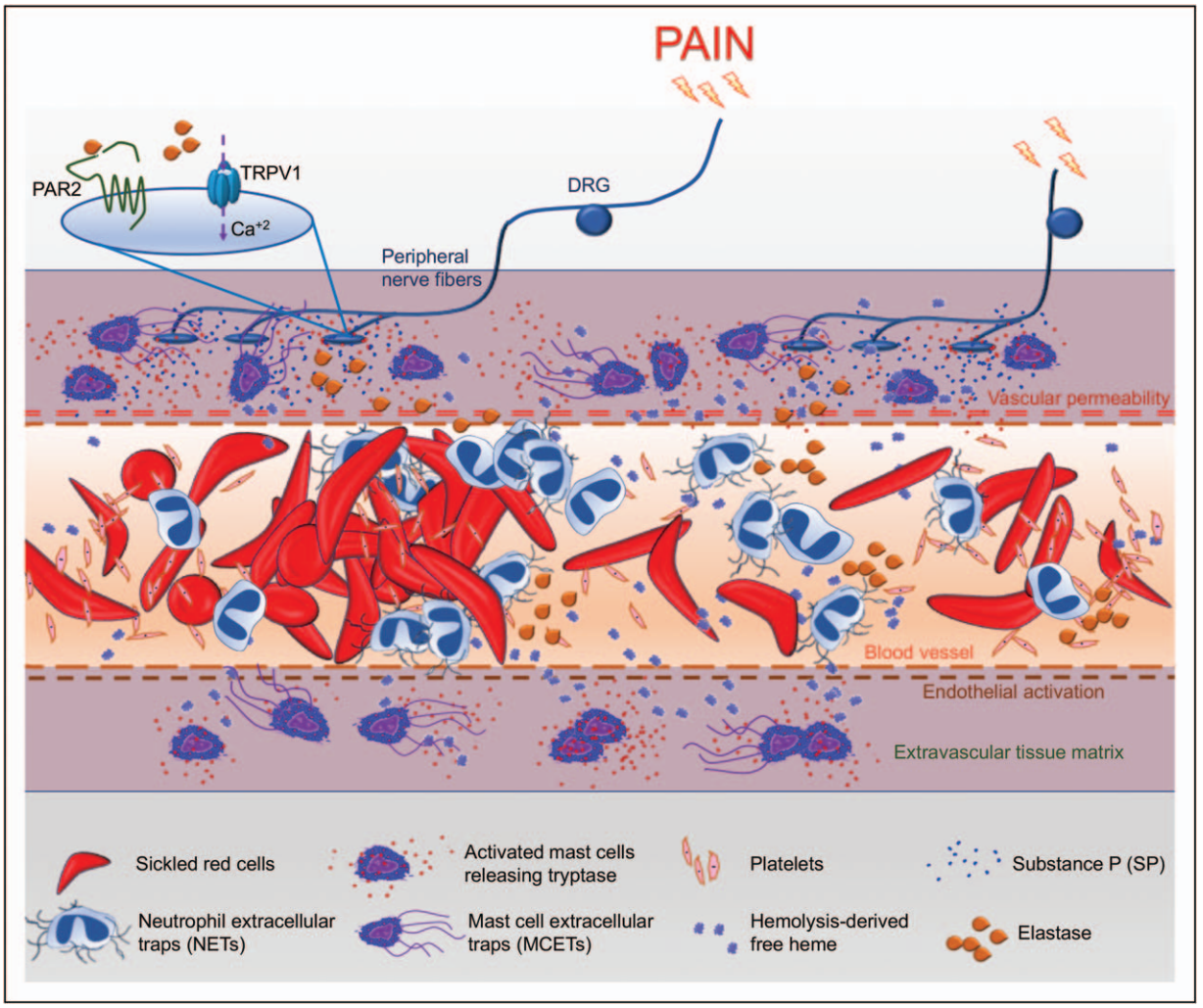

FIGURE 1. Neurovascular and neuroimmune mechanisms of pain in SCD: Complex multicellular interactions and inflammatory and noxious microenvironment surrounding the peripheral and central nervous system underlie pain in SCD. *DRG*, dorsal root ganglion; *MCETs*, mast cell extracellular traps; *NET*, neutrophil activation and extracellular trap; *RBCs*, red blood cells; *SCD*, sickle cell disease; *SP*, substance P; *VOC*, vasoocclusive crises.

below. Noxious environment including nerve injury stimulates the primary afferents to transmit action potentials to dorsal root ganglion (DRG) neurons and their subsequent amplification to the second-order neurons in the dorsal horn of spinal cord, where they undergo modification before being relayed to the brain. Repeated episodes of VOC may lead to the sensitization of peripheral and central nervous system (CNS) neurons, which may lead to a hyperalgesic state and impair response to therapy. Constitutive peripheral and central sensitization have been observed by us in Berkeley sickle mice with chronic hyperalgesia [34,35], and in chronic pain patients with SCD [36]. Moreover, polymorphisms within diverse genes including the glucocorticoid receptor (nuclear receptor subfamily 3 group C member 1), GTP cyclohydrolase 1, and the transient receptor potential (TRP) family member, TRPA1, influence acute pain crisis and pain heterogeneity in SCD [37–39]. Additionally, cold and mechanical pain was found to be significantly worse during acute painful events compared with resting states (baseline) in young patients with SCD [40], whereas older age was associated with lower cold, heat, and mechanical threshold for pain [41]. Nonetheless, it is likely that recurrent episodes of acute pain lead to a sustained noxious environment replete with an inflammatory state and oxidative stress, causing the CNS to remain at a continuous hyperexcitable state leading to the transition from acute to chronic pain.

CHRONIC PAIN IN SICKLE CELL DISEASE

Chronic pain occurs in a significant number of SCD patients [42–44]. Additionally, many sickle patients

suffer from life-long chronic pain [8]. Hydroxyurea treatment, reduced infection, and early screening have improved survival of patients with SCD [16,42,45]. Hydroxyurea treatment reduces the number of acute crises per patient year, but it does not alleviate pain on a daily basis. With patients living longer, chronic pain may further increase with age, which necessitates opioid use.

Hematopoietic stem-cell transplantation (HSCT) is a successful curative therapy for SCD resulting in disease-free survival in over 85% of patients [46,47]. After successful HSCT, most patients were weaned off opioids; however, a subgroup of patients (about 40%) who suffered from chronic pain prior to HSCT continued to experience pain that required opioid treatment [48]. The results indicate that preexisting neurochemical or neuropathic alterations from chronic pain may not be resolved post-HSCT. HSCT may therefore need to be performed at an early age to provide complete remission of pain [8].

NEURAL-IMMUNE AXIS IN THE GENESIS OF CHRONIC PAIN

Proteases released from inflammatory cells have the potential for activating the nociceptors on nerve fibers (Fig. 1). We describe evidence from two different proteases released from mast cells and neutrophils in SCD for their contribution in pain.

Contribution of mast cells

Mast cells are tissue-resident granulocytes well known for their contribution to allergic response [49]. Of relevance to SCD, mast cells are located in the vicinity of the vasculature and nerve fibers. In our preclinical studies, we have identified mast cells as a critical contributor to sickle pathobiology and sickle pain [50▪▪]. In sickle mice, incitement of hypoxia/reperfusion injury to simulate acute pain in SCD led to the increased release of the neurotransmitter substance P and calcitonin gene-related peptide (CGRP) in the skin, blood, and DRG [50▪▪,51,52]. Our data suggest that activated mast cells in the skin of transgenic sickle mice release tryptase which stimulates release of substance P and CGRP from peripheral nerve endings via activation of protease-activated receptor 2 (PAR2) and TRP cation channel, subfamily V, member 1 (TRPV1) (Fig. 1) [50▪▪]. TRPV1 has been shown to mediate pain in sickle mice [53]. Moreover, inhibition or blockade of proinflammatory C–C motif chemokine receptor 2-alleviated mechanical and cold hypersensitivity in sickle mice and also prevented responsiveness of sensory neurons to an agonist of TRPV1 [54]. Substance P-mediated plasma extravasation and leakage lead to neurogenic inflammation, reinforcing endothelial dysfunction and the release of additional inflammatory mediators that further sensitize the peripheral nociceptors. Substance P can further activate mast cells leading to a feed-forward loop that perpetuates within the sickle microenvironment and contributes to sustained peripheral sensitization and pain [50▪▪]. In clinical observations, sickle patients exhibited elevated circulating tryptase and substance P levels compared with healthy controls [55–57]. Additionally, release of glial fibrillary acidic protein (GFAP) has been observed in patients with SCD during VOC – thus indicating the presence of neuroinflammation in SCD [58]. Imatinib, an inhibitor of c-kit (required for mast cell activation), was shown to be effective in reducing the occurrence of VOC in SCD patients with acute myeloid leukemia [59–61]. Importantly, we found that imatinib reduced both chronic and acute hypoxia/reoxygenation-evoked hyperalgesia in sickle mice [50▪▪]. Cromolyn sodium (a mast cell stabilizer) alone did not reduce hyperalgesia in sickle mice but rather potentiated the analgesic effects of suboptimal morphine dosage. Both imatinib and cromolyn sodium reduced neurogenic inflammation in the skin, and systemic concentrations of mast cell products, including tryptase, neuropeptides substance P and CGRP, and inflammatory cytokines in sickle mice [50▪▪]. Thus, targeting mast cell activation presents a promising avenue for sickle pain therapeutics.

Mast cells may also have a more direct impact on sickle pain via disrupting neurovascular architecture within the SCD microenvironment. Previously, we demonstrated evidence of structural disarray in peripheral nerves and vasculature in the skin of sickle mice coexistent with increased immunoreactivity of substance P and CGRP [62]. We recently observed mast cell extracellular traps (MCET) colocalized with high tryptase immunoreactivity in the skin of sickle mice, which penetrated the surrounding vasculature, indicating a direct role of mast cells in vascular permeability and endothelial dysfunction (Fig. 1) [33]. Additionally, these traps entangled axonal nerve fibers projecting axonal sprouting analogous to nerve injury often seen in neuropathic pain conditions. Prepriming mast cells *in vitro* with TNF-α (to simulate inflammation) and subsequently treating with heme resulted in MCET formation in most mast cells derived from sickle mice (but in fewer mast cells from control mice) [63]. Mast cells, when activated, release inflammatory molecules, which are transported to neighboring cells via exocytosis through direct contact, via extracellular vesicles, through extended nanotubes or MCETs. Thus, MCETs can have both direct and indirect impact on the neurovascular interaction in SCD.

Contribution of neutrophils

The presence of NETs and neutrophil activation has been associated with SCD – both in sickle patients and in-vivo studies using transgenic sickle mice [64–66]. NETs release elastase, a proteolytic enzyme, which contributes to neuropathic pain via activation of PAR2 and TRPV4 to elicit peripheral sensitization [67]. Plasma elastase levels, possibly released from activated neutrophils, are elevated in sickle patients compared with healthy individuals and are further increased during VOC [64]. Although vascular perturbations resulting from leukocyte activation have been implicated in the pathogenesis of VOC, the potential role of elastase in directly affecting sickle pain is a novel finding from our laboratory [68]. We have found that regulation of proteases including elastase is impaired in SCD. Serine protease inhibitor A (Serpin) A3N and A1e [genes encoding α-1-antitrypsin (A1AT), an endogenous inhibitor of elastase] are downregulated in the DRG of sickle mice compared with control mice [69▪▪]. Treating sickle mice with sivelestat, a molecular inhibitor of elastase, reduced chronic hyperalgesia and elastase activity in DRG [70]. Additionally, sickle mice treated with FDA approved human A1AT/Prolastin-C, evinced significantly reduced thermal hyperalgesia [71]. This indicates that elastase contributes to central mechanisms of sickle pain. Interestingly, high levels of A1AT in sickle patients have been recently shown to be positively associated with hemolytic and inflammatory parameters with negative correlation with renal parameters – suggestive of a regulatory role of SerpinA genes [72]. Additionally, A1AT deficiency is known to induce acute lung injury [73]. We have found increased activity of elastase in the lungs of sickle mice, which was attenuated with prolastin-C treatment. Thus, pain remission in sickle mice treated with A1AT may be a sum of influences involving contributions from improving known pulmonary complications and indicators in SCD.

CENTRAL SENSITIZATION: A CRITICAL PLAYER IN THE PATHOGENESIS OF CHRONIC PAIN AND RESPONSE TO THERAPY IN SICKLE CELL DISEASE

Sickle patients suffering from pain with longer hospital stays exhibit higher pronociceptive and lower antinociceptive default mode network connectivity of the brain [74]. Similarly, sickle patients had higher activity in pain-processing regions of the brain compared with control patients, which is indicative of a hyperexcitatory state of pain-processing circuitry in SCD [74–76]. Sickle patients exhibit heightened perceptions of cold and hot hyperalgesia, indicating active central sensitization [41]. In sickle mice, we observed enhanced excitability in spinal dorsal horn neurons suggestive of central sensitization, in addition to activation of proneuroexcitatory mitogen-activated protein kinase signaling pathways in the spinal cord of sickle mice [35]. In the spinal dorsal horn, we observed increased microglial and astrocyte activation associated with elevated reactive oxygen species (ROS), substance P, and GFAP [77]. Treatment with coenzyme Q10 (CoQ10) reduced VOC in sickle patients with indication of reduced ROS [78]. Moreover, children with SCD were reported to have higher plasma GFAP compared with healthy patients [58]. In sickle mice, treatment with CoQ10 and curcumin resulted in reduced hyperalgesia associated with reduced activation of microglial cells and astrocytes, and decreased levels of substance P, ROS, and GFAP in the spinal cord, suggesting that central mechanisms of sickle pain are mediated by spinal oxidative stress and elevated neuroimmune interactions. Interestingly, electroacupuncture in sickle mice showed a variable response to analgesia, with high, medium, and no response [79▪]. High responders showed lower spinal substance P, whereas low responders showed high substance P. Thus, heterogeneity in pain and response to therapy may depend upon central sensitization.

STRATEGIES TO DEVELOP TARGETED THERAPIES

Strategies targeting pain signaling pathways, neurotransmitters, and analgesic receptors such as Ca^{2+}/calmodulin-dependent protein kinase II [80,81], protein kinase C delta (PKCδ) receptor on GABAergic neurons [82▪▪], nociceptin opioid receptor [51], and cannabinoid receptors [52,62] have shown promise in relieving pain in preclinical animal models and are under investigation in clinical trials (*discussed in details elsewhere*) [6▪,7]. Although targeting the mechanisms underlying VOC and peripheral and central mediators of pain may provide adjuvant pain therapeutics, an important question remains unanswered: *why do post-HSCT patients, who have had prior conditions of chronic pain, continue to suffer from chronic pain in the absence of vascular sickle pathophysiology?* This phenomenon indicates a constitutive alteration in the pain-neuronal circuitry of sickle patients with chronic pain. In sickle mice, nerve damage is evident from our earlier studies [62]. Recently, we found that the nerve-regeneration associated gene, small proline-rich protein 1A (SPRR1A), is downregulated in the DRGs of older mice (5 months old) compared with younger mice (2 months old) [68]. SPRR1A has been shown to be involved in nerve regeneration in response to spared

nerve injury [83,84]. Therefore, an impairment in nerve repair because of reduced SPRR1A may contribute to a diminished capacity to respond to persistent nerve injury and may underlie the initiation and continuation of chronic pain. The presence of similar prolonged genetic alterations could explain the chronic pain-experienced post-HSCT by patients even in the absence of vascular sickle pathophysiology. Future efforts should concentrate on the identification of modulators driving the transition from acute to chronic pain in sickle individuals as age progresses.

PERCEPTION-BASED STRATEGIES TO TARGET PAIN IN SICKLE CELL DISEASE

Neurovascular physiological findings in patients with SCD showed increased autonomic nervous system (ANS) reactivity [85]. Patients demonstrated immediate vasoconstriction in anticipation upon being told that a painful stimuli will be applied, prior to the application of the actual stimuli, suggesting that perception of pain is in part derived from the activation of ANS [85]. Biophysical markers of vascular reactivity in real time may provide an objective measure of response to pain in SCD and need to be developed further [86]. In sickle mice, we observed that companionship of male mice with females reduced hyperalgesia in males with a concomitant increase in serotonin in the spinal dorsal horn and rostral ventromedial medulla region of the brain, suggestive of the contribution of spinothalamic pain inhibitory pathway in SCD [87]. Therefore, modulation of ANS and perception of pain through the higher brain centers using integrative nonpharmacologic approaches, including mindfulness, hypnosis, and lifestyle changes, may have a beneficial effect on moderating pain and reduce opioid use [88].

DIET-FOCUSED STRATEGIES TO REDUCE SICKLE PAIN

Studies investigating the ability of L-arginine and L-glutamine supplementation to reduce acute VOCs suggest that diet could significantly impact SCD pain [28,29]. In fact, deficiencies of many nutrients, including vitamins, zinc, magnesium, and fatty acids, are well documented in SCD [89]. In a recent study, we found that high-calorie sickle diet supplemented with a combination of protein and multiple micronutrients resulted in significantly increased survival of neonatal sickle mice into adulthood compared with controls fed a regular diet, and interestingly, male pups' survival improved with feeding of parents with high-calorie sickle diet [90▪]. Moreover, this diet along with companionship (male sickle and female mice housed together) resulted in significantly reduced hyperalgesia in males, via serotonergic descending pain inhibitory pathway [87]. Thus, protein-rich micronutrient-supplemented high-calorie diet may offer improved pain relief, which merits further investigation.

CONCLUSION

Sickle pain is often intractable requiring opioids on a daily basis. Heterogeneity in peripheral and central activation of nociceptive mechanisms evoked by a multitude of pathogenic events in the periphery as well as in the CNS because of variable noxious insult of different intensity and regional variability may underlie the occurrence of differences in pain frequency, persistence, and response to therapy. Mechanism-based therapeutics for reducing acute VOC have shown promise. However, the multifaceted pathobiology of SCD warrants the discovery of objective mechanism-based biomarkers for targeting appropriate therapies. Recognition of subsets, who would show response to specific preventive or analgesic therapies, is required to achieve improved analgesic outcomes. Affective modulation of pain with integrative approaches requires more attention to improve analgesic outcomes without inadvertent effects of opioids.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
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