We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Mechanisms of Pain in Sickle Cell Disease

Anupam Aich, Alvin J Beitz and Kalpna Gupta

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/64647>

Abstract

Pain is one of the most common features of sickle cell disease (SCD) lacking effective therapy. Pain in SCD is relatively more complicated than other conditions associated with pain requiring understanding of the pathobiology of pain specific to SCD. The characterization of pain to define the diverse modalities of nociception in SCD is currently under progress via human studies accompanied by transgenic mouse models of SCD. Sickle pathobiology characterized by oxidative stress, inflammation and vascular dysfunction contributes to both peripheral and central nociceptive sensitization via mast cell activation in the periphery, and reactive oxygen species and glial activation and endoplasmic reticulum stress in the spinal cord among other effectors. These effects are mediated via several cellular receptors, which can be targeted to produce positive therapeutic outcomes. In this chapter, we will discuss the present understanding of molecular mechanisms of SCD pain and outline the mechanism-based translational potential of novel actionable targets to treat SCD pain.

Keywords: pain, sickle cell disease, neurogenic inflammation, substance P, mast cell

---

1. Introduction

Pain is a hallmark feature of sickle cell disease (SCD), which can start in infancy, leading to hospitalization, reduced survival and poor quality of life. Pain in SCD is unique because of unpredictable and recurrent episodes of acute pain due to vaso-occlusive crises (VOC), in addition to chronic pain experienced by a majority of adult patients on a daily basis [1]. Treatment choices remain limited to opioids, which impose liabilities of their own including constipation, mast cell activation, fear of addiction and respiratory depression [2]. Moreover, significantly larger doses of opioids are required to treat pain in SCD as compared to other acute and chronic pain conditions [1]. Pain can be lifelong in SCD and may therefore influence

cognitive function and lead to depression and anxiety, which can in turn promote the perception of pain [1].

Treatment of chronic pain remains unsatisfactory overall, perhaps due to the diverse pathobiology in different diseases. Therefore, it is critical to understand the mechanisms specific to the genesis of sickle pain to develop targeted therapies. Vascular dysfunction, inflammation, ischemia/reperfusion injury and oxidative stress in the wake of VOC can each evoke activation of the nociceptive nerve fibers leading to acute pain. On the other hand, constant endothelial activation, inflammation and reactive oxygen species (ROS) generation may underlie the nerve injury leading to chronic inflammatory and/or neuropathic pain. Endothelial activation, inflammation and oxidative stress have been extensively characterized in the periphery [1] but not in the central nervous system in SCD. Both peripheral and central mechanisms may underlie the nociceptor activation leading to pain. In this chapter, we describe the sickle pathobiology that may contribute to pain and define possible treatable targets.

1.1. Presentation of pain in SCD

Current research in characterizing pain in SCD patients indicates that both acute and chronic pain are prevalent among the adult patients, while infants and children mostly suffer from acute pain [3–5]. The shift from acute to chronic pain may therefore occur during the transition from childhood to adolescence. Young children with a median age of 3.8 years (range 0.3–7.6 years) exhibited less frequent pain, occurring on 1.6% of a total of 141,197 days [3]. Yet, only 14% of these episodes required hospitalization, and infants between the age of 0 and 12 months had the most pain (80%) associated with dactylitis [3]. In another study on 100 young subjects, about 40% of children and adolescents in the age range of 8–18 years reported chronic pain with another 40% exhibiting episodic pain, and the remainder had no pain [4]. Though the pain intensity and quality of life were comparable among the young patients with chronic and episodic pain, the patients with chronic pain suffered from greater functional disability, depression and hospital admissions compared to the episodic pain group [4]. The adult patients recruited in the Pain in Sickle Cell Epidemiology Study (PiSCES) reported chronic SCD pain on 54.5% of 31,017 days at home [5]. Opioids have remained the major strategy to treat acute sickle pain, while chronic pain is managed with the combination of non-steroidal anti-inflammatory drugs (NSAIDs), opioids, anti-depressants and anticonvulsant medications [6]. However, to date no satisfactory therapy exists.

2. Characteristics of pain in SCD

Based on transgenic mouse models of SCD and presentation of pain in patients, four major characteristics of pain have been described (**Table 1**). These characteristics include increased sensitivity to (i) mechanical, (ii) heat and (iii) cold stimuli and (iv) decreased grip force ([3, 7–12, 14–17, 22–27], Lei et al., 2016, under review). Characterization of SCD pain in patients has been quite challenging due to the episodic and sudden nature of the acute pain, often requiring hospitalization. The characterization of chronic pain is challenging owing to the complex and

Characteristics of pain	Pain phenotyping method	
	Subjects with SCD	Transgenic mice expressing sickle hemoglobin
Mechanical hy- peralgesia	QST—tactile sensation using a cotton ball, hand-held soft brush and non-penetrating pin-pick on the left/right forearm [7] and reported via visual analog scale QST—using von Frey filaments at the thenar eminence of non-dominant hands and lateral dorsum of randomly selected foot [8]	Paw withdrawal responses to von Frey monofilaments in NY1DD & S+S ^{antilles} mice [9], in BERK mice [9–13] and in Townes mice [Lei et al., communicated]
Heat hyperalgesia	QST—using Thermal Sensory Analyzer (Medoc: Israel) which employs cold temperature to the skin via a peltier-based thermode [7, 8]	Paw withdrawal latency and frequency in response to static heat stimuli in BERK mice [9, 11–13] and in Townes mice [14], Lei et al., 2016, under review]
Cold hyperalgesia	QST—using Thermal Sensory Analyzer (Medoc: Israel) which employs cold temperature to the skin via a peltier-based thermode [7, 8, 15] Case-crossover study and retrospective statistical analysis of occurrence of pain/VOCs in relation to weather conditions [16–20]	Paw withdrawal latency and frequency in response to static cold stimuli in S+S ^{antilles} mice [9], in BERK mice [9, 10, 12, 13] and in Townes mice [[14], Lei et al., 2016, under review] Temperature preference assay for BERK mice [21]
Deep-tissue/ musculoskeletal hyperalgesia	Questionnaire-based assessment using the Nordic musculoskeletal symptoms questionnaire and the SF-36 Health Survey [22]	Tensile force of peak forelimb exertion measured using grip force meter—in NY1DD & S+S ^{antilles} mice [9], in BERK mice [9–12] and in Townes mice [Lei et al., 2016, under review]
Observer-based Quantification	Not documented	Observer-based quantification of facial expression measured by action units and body parameters from changes in the back curvature [23]

Table 1. Characteristics of pain in SCD.

intractable nature of SCD pain, which may have a combination of inflammatory, nociceptive and/or neuropathic origin. Clinical studies have used the patient-reported questionnaire-based assessment and quantitative sensory testing (QST) approaches to evaluating the nature and characteristics of pain in patients [7, 15, 28–30]. In a recent QST of 48 children with SCD, 13 individuals exhibited increased mechanical allodynia and also decreased sensitivity to heat or cold detection (hypoesthesia) [7]. A similar study of 27 SCD patients aged 10.3–18.3 years with race-matched control patients corroborated the heat-cold sensation features but demonstrated an increased cold-pain feature in SCD patients [15]. In contrast, Brandow et al. [8].

found a decreased threshold for cold and heat detection in a cohort of 55 SCD patients (≥ 7 years old) compared to 57 race-matched healthy controls [8]. In contrast, no significant differences were observed in these patients in response to mechanical stimuli [8]. Cold hypersensitivity under cold weather conditions has been found to be associated with pain and VOC in pediatric [18]. and adult patients [19, 20]. Musculoskeletal/deep-tissue pain has been found to be present at multiple sites including the arms, chest and lower back in a questionnaire-based study of 27 adult patients with mean age of 31.77 years [22].

In parallel, transgenic mouse models expressing human sickle hemoglobin, which mimic the SCD pathobiology and pain, have been highly instructive in developing the understanding of sickle pain [9]. Transgenic sickle mouse models have been able to recapitulate the features of SCD with variable severity depending upon the extent of expression of human sickle hemoglobin (HbS) and the presence/or absence of mouse hemoglobin α and β [31]. NY1DD sickle mice developed by Fabry et al. contain a single copy of the human α and β^s transgene with deletion of mouse major β genes, but express mouse α chains and express about 26% HbS leading to a mild phenotype [32]. S+S^{antilles} mice carry an additional mutation and express about 42% of human β^s showing a stronger phenotype than NY1DD mice [33]. These mice with milder pathology do not show significant characteristics of chronic or acute pain [9], which can be induced by hypoxia/reoxygenation. On the other hand, homozygous Townes [34] and Berkeley (BERK) [35] transgenic mice express exclusively human α and β hemoglobins without mouse α or β chains and express $>99\%$ human HbS. Consequently, these mice demonstrate a severe SCD phenotype including excessive hemolysis, inflammation, organ damage and shorter life span [26, 31, 34–37]. BERK and Townes models show constitutive chronic hyperalgesia early in life ([12], Lei et al., 2016, under review). Moreover, hypoxia/reoxygenation treatment evokes a further increase in hyperalgesia simulating acute pain during VOC, compared to their specific background strains expressing normal human hemoglobin A ([12], Lei et al., 2016, under review). Therefore, BERK and Townes homozygous sickle mice exhibit human sickle pathology as well as pain similar to patients with SCD. Hence, both of these models are well suited to understand how sickle pathobiology leads to the genesis and progression of pain in SCD recalcitrant to therapy.

3. Sickle pathobiology underlying pain

Sickling of RBCs under low oxygen due to a point mutation in the beta hemoglobin chain of hemoglobin is the primary pathogenic condition in SCD [13]. Sickle RBCs have impaired oxygen-carrying ability and cause jamming of micro-capillaries via adhesion to endothelial walls in the event known as VOC [21]. Resultant SCD pathobiology is characterized by inflammation, oxidative stress, ischemia reperfusion injury and organ damage [21], all of which can independently and/or cumulatively lead to activation of the nociceptive system (**Figure 1**). For example, the increased levels of inflammatory cytokines, such as TNF α and IL-6 [38] in the periphery and the central nervous system (CNS) can activate nociceptors and spinal nociceptive neurons, which may in turn be an outcome of activated macrophages or mast cells in the periphery and glial cells in the CNS driving a vicious cycle of inflammation

and pain (**Figure 1**). Decreased oxygenation and reduced blood supply due to vascular occlusion during VOC may impair oxygenation and nutrient supply to the nerve fibers, thus causing nerve damage and activation of nociceptors. Hematologic, inflammatory and vascular dysfunctions have been well characterized in the periphery, but not in the CNS in subjects with SCD and in sickle mice [21, 39]. Our laboratory demonstrated oxidative stress, increased inflammatory cytokines and neuropeptides in the spinal cord of sickle mice as compared to control mice [12, 40]. Thus, sickling of RBCs affects the periphery and the CNS, which may lead to a complex pathobiology of pain in SCD leading to inflammatory, nociceptive and neuropathic pain. SCD is also characterized by phenotypic heterogeneity and unpredictable episodes of VOC, which may vary in frequency, recurrence and intensity among patients [21]. Therefore, SCD pain displays a marked heterogeneity in the context of neurobiology.

Figure 1. Sickle pathobiology evoked peripheral and central mechanisms of pain: Sickle pathobiology comprising vaso-occlusive crises, hypoxia/reoxygenation injury, hemolysis, inflammation and organ damage can sensitize nerve fibers in the periphery. Activated mast cells release neuropeptide substance P (SP) and other mediators in the skin further sensitizing peripheral nociceptors. Pain signals are transmitted from periphery through dorsal root ganglion (DRG) and spinal cord to the brain. Increased reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress, inflammatory milieu, glial activation accompanied by increased toll-like receptor 4 (TLR4) phosphorylation of p38MAPK with correlative nociceptor sensitization in the spinal cord of sickle mice suggest persistent central sensitization. Sustained and enhanced central sensitization contributes to antidromic release of neuropeptides and nociceptive mediators in the periphery, which in turn accentuates peripheral nociception without noxious stimuli. Thus, a vicious feed-forward cycle of peripheral and central sensitization continues and chronic pain persists in sickle pathobiology.

4. Peripheral and central mechanisms of pain in SCD

Transgenic mouse models described above have been highly instructive in examining the mechanisms specific to sickle pain. Pain can be both chronic as well as acute following VOC and the underlying mechanisms may or may not vary between the two. BERK sickle mice show significantly higher chronic hyperalgesia as compared to age- and gender-matched Townes sickle mice (Lei et al., 2016, under review). Most of the mechanisms have been examined in BERK sickle mice for both chronic hyperalgesia constitutively existent in these mice and acute pain following hypoxia/reoxygenation to simulate VOC [9]. Structural analysis of the skin of homozygous BERK mice (expressing human sickle hemoglobin) compared to control mice (expressing normal human hemoglobin) showed alterations in nerve fibers and blood vessels [12]. Vascular and nerve plexi as well as normal branching is diminished in BERK sickle mice skin, showing nerve sprouting indicative of inflammatory and neuropathic pain [12]. These structural changes are accompanied by increased expression of neuropeptides substance P (SP) and calcitonin-gene-related peptides (CGRP) in the skin [12]. Concomitantly, skin in BERK sickle mice is significantly thinner with a comparatively thinner epidermis, similar to that observed in other murine models of pain such as diabetes [41]. These structural and neurochemical alterations in association with well-known inflammatory milieu may likely activate nociceptors on the peripheral nerve terminals as demonstrated by activation of transient receptor potential cation channel subfamily V member 1 (TRPV1) in the skin of BERK sickle mice [11]. This peripheral nociceptor activation leads to the activation of glial cells and neuronal activating transcription factor 3 (ATF3) in the dorsal root ganglion (DRG) [10], which may lead to the transmission of increased action potentials to the second-order neurons of the spinal cord. Indeed, second-order neurons in the dorsal horn of the spinal cord show constitutive nociceptor sensitization in electrophysiological recordings in the BERK sickle mice [42]. Nociceptive neurons in the dorsal horn of sickle mice show increased excitability and an increased rate of spontaneous activity [42]. These electrophysiological responses are accompanied by higher response to mechanical stimuli and prolonged after-discharges following the mechanical stimulus, suggestive of central sensitization [42]. This sustained and continuous activation of spinal neurons may lead to increased release of neuropeptides and nociceptive mediators, which may be released into the periphery antidromically, in turn activating the peripheral nerve terminals without noxious insult. This vicious feed-forward cycle of peripheral and central sensitization may underlie chronic pain recalcitrant to therapy. Also, increased phosphorylation of mitogen-activated protein kinases related to neuronal hyper-excitability is supportive of central sensitization in sickle mice [42]. Concurrently, Darbari et al. evaluated brain connectivity in 25 adolescent and young patients using functional magnetic resonance imaging (fMRI), and these patients were divided into low and high pain groups based on their hospitalization frequency [25]. In the fMRI analysis, the high pain group exhibited excessive pronociceptive connectivity while the low pain group displayed greater association with brain regions implicated in anti-nociception [25]. In this study, although all the patients were on hydroxyurea, the expression of fetal hemoglobin (HbF) was higher in the low pain group and was in positive correlation with anti-nociceptive connectivity [25]. These results suggest involvement of central mechanisms in sickle pain. Moreover, central sensitization in sickle

patients was recently evaluated using QST, questionnaires and daily pain diaries [29]. Those patients with higher scores for central sensitization exhibited worse manifestations of SCD. Therefore, understanding the molecular mechanisms that drive peripheral nociceptor and central nociceptive neuronal activation is cardinal to developing effective therapies.

We found that mast cells, a tissue-resident granulocyte, are activated in the skin of sickle mice and contribute to neurogenic inflammation, inflammation and pain [43]. Mast cells from sickle mouse skin show significantly higher transcripts for toll-like receptor 4 (TLR4) as compared to mast cells from control mice [43]. Moreover, heme, the product of excessive hemolysis, a significant feature of SCD, can activate mast cells in the periphery. Additionally, spinal TLR4 expression and cell-free heme are significantly higher in sickle mice compared to control mice (Lei et al., under preparation). It has been shown that excess heme can induce spinal microglial activation via TLR4 *in vitro* [44], and thus, this may be a mechanism contributing to central sensitization in sickle patients. In this regard, spinal microglial activation is suggested to be a contributor to central sensitization leading to pain [45]. Spinal microglial and astroglial activation is correlative to increased ROS production and SP in the spinal cord of sickle mice [40]. Spinal microglial activation and ROS production via TLR4 can also be an accessory to the central sensitization process [44]. Most of these studies were performed in male mice. Recently, Sorge et al. have demonstrated that nerve injury-induced pain in male mice (not in female mice) are mediated via TLR4 (possibly via microglial activation) [46], but via T-lymphocytes instead of microglial cells in female mice [47]. Though the PiSCES report (from extensive multi-center human study on sickle pain) found no significant difference in pain sensation and intensity according to gender differences [48], it is yet to be demonstrated/verified whether sickle pain is mediated via gender-specific pathways.

Peripheral injury due to acute VOC evokes acute pain, but it is likely that the chronic inflammatory state, oxidative stress, vascular dysfunction and nerve injury lead to sustained sensitization of both peripheral and central nociceptive neurons. SCD pain can also be of neuropathic origin, which has been demonstrated in patient-reported [49, 50] and QST-based studies [30]. Circulating glial fibrillary acidic protein (GFAP) and SP expression are significantly higher in subjects with SCD as compared to normal healthy subjects [51, 52]. In a group of 2–18-year-old SCD patients, serum SP levels were found to be elevated, which increased further during VOC [52]. SP possibly acts on neurokinin 2 (NK2) receptors to sensitize TRPV1 leading to an enhancement of afferent excitability and an increase in peripheral nociception [11]. SP can further contribute to plasma extravasation due to its vasodilatory effect leading to neurogenic inflammation, in addition to activating mast cells [43, 53]. The painful dactylitis in children with SCD [3] may be due to neurogenic inflammation in response to increased release of SP from the peripheral nerve terminals. Increased GFAP has been associated with stroke in children with SCD and supports increased glial cell activity observed in the DRG and dorsal horn of the spinal cord of sickle mice [12, 40, 51]. Zappia et al. found that cold hyperalgesia in sickle mice increases with age [54], and these data are in accord with the finding that sickle patients experience increased thermal hypersensitivity as they age [8]. Additionally, the expression of endothelin 1 and tachykinin receptor 1 were increased by 2.7- and 1.6-fold, respectively, in the DRG of sickle mice, compared to control mice [54]. Endothelin 1 may

contribute to cold hyperalgesia via endothelin receptors [55], and SP can contribute to hyperalgesia via tachykinin 1 [56] located in the peripheral nervous system. These findings suggest that diverse SCD pathobiology underlies the genesis and progression of recalcitrant pain in SCD. Therefore, multimodal targeting may be required in a case-specific manner to achieve satisfactory analgesic outcomes.

5. Treatable targets for ameliorating sickle pain

5.1. Opioid receptors (ORs)

The current mainstay of treatment for acute and chronic pain in SCD is opioids. To assess opioid effects on chronic SCD pain in adult patients, 15,778 home pain days of 219 patients were monitored [57]. On 78% of the pain days, the patients used opioids—38% of the total patients used long-acting opioids and 47% used short-acting opioids. The striking outcome of this study was that the opioid usage significantly correlates with the severity of pain intensity and other manifestations of SCD—suggestive of negative impact of the opioids on the pathophysiology of chronic SCD [57].

Although the analgesic action of morphine is vital for pain remission, the effects of morphine can be multifactorial leading to opioid-induced hyperalgesia [58] and possible exacerbation of other complications of SCD [2]. Morphine exacerbates renal pathology in sickle mice [59], and its interaction with TLR4 may promote neuroinflammation [60]. Morphine-induced angiogenesis and co-activation of receptor tyrosine kinases may influence organ pathology including retinopathy, nephropathy, stroke and pulmonary arterial hypertension [2].

Among four different opioid receptors, mu opioid receptor (MOR) facilitates analgesic action of opioids [2]. Repeated activation of MORs can lead to tolerance to opioids. Morphine transactivates platelet-derived growth factor receptor—beta (PDGFR- β) [61]—and inhibition of PDGFR- β by imatinib (a tyrosine kinase inhibitor) attenuates morphine tolerance [62]. Reversal of tolerance to morphine by Imatinib can also be a consequence of reduced activation of mast cells as discussed below. Therefore, strategies to ameliorate the side effects and reduce tolerance are required to optimize pain control with opioids.

Nociceptin opioid receptor (NOP/OR) is another member of opioid receptor family which contributes to nociceptive signaling [63]. The endogenous ligand of NOP/OR is nociceptin/orphanin FQ (N/OFQ), and it is known to attenuate secretion of neuropeptides (SP and CGRP) from peripheral nerve endings [64] and from mast cells [65]. Our recent findings demonstrate that a small molecule agonist of NOP/OR, AT200, is able to decrease hyperalgesia in sickle mice by reducing inflammation and mast cell activation [66]. Continuous treatment of sickle mice with AT200 did not produce any tolerance, suggestive of a feasible opioid drug devoid of tolerance. This approach of targeting other ORs with potential to attenuate underlying sickle pathobiology needs to be investigated further.

5.2. Mast cells

Mast cells are tissue resident granulocytes, well known for their role in pruritis and anaphylaxis [67]. We (Gupta et al.) found that mast cell activation contributes to pain in sickle mice [43]. Constitutive mast cell activation leads to inflammation characterized by the release of inflammatory cytokines in the skin and neurogenic inflammation in sickle mice. Cromolyn sodium, a mast cell stabilizer, and imatinib, an inhibitor of mast cell c-kit, attenuated these mast cell associated effects in mice [43]. Neurogenic inflammation characterized by excessive plasma leakage from the vasculature in response to SP released from the nerve terminals is reminiscent of painful dactylitis in children with SCD. Activated mast cells release tryptase, which activates protease-activator receptor 2 (PAR2) on peripheral nerve endings stimulating the release of SP [43]. In turn, SP then stimulates vascular leakage and vasodilation as well as further activation of mast cells, leading to a vicious cycle of inflammation, neurogenic inflammation and hyperalgesia [43]. Pharmacological and genetic inhibition of mast cells contributes to reduction in sickle pain in mice [43].

Morphine is an activator of mast cell degranulation [67]. Sickle mice pre-treated with cromolyn or imatinib show increased analgesic response to a sub-optimal dose of morphine [43]. It is therefore likely that morphine acts on the CNS to induce analgesia but promotes hyperalgesia by simultaneously activating mast cells, resulting in reduced analgesic efficacy. Therefore, co-treatment strategies with mast cell stabilizers or imatinib may improve analgesic outcomes and reduce tolerance (as discussed above) and may even minimize the side effects of opioids.

Products released from activated mast cells include SP, cytokines and growth factors, such as PDGF and VEGF, which can directly act on the vasculature in the vicinity [67]. We have recently observed that mast cell-derived mediators cause increased permeability in monolayers of mouse brain microvascular endothelial cells by stimulating endoplasmic reticulum (ER) stress [Luk et al., communicated]. Additionally, ER stress has been shown to mediate pain in diabetic neuropathic rats [68]. Thus, inhibiting mast cells in combination with ER stress inhibitors may have an impact on endothelial dysfunction and pain—two critical characteristic features of SCD. Therefore, common targets influencing vascular, inflammatory and nociceptive mechanisms may provide comparatively more effective treatable targets that reduce pain, inflammation and vascular complications without inadvertent effects on SCD.

5.3. Cannabinoid receptors (CBRs)

Cannabinoid receptors (CBRs) CB1R and CB2R are 7-transmembrane G-protein coupled receptors, expressed in the CNS, as well as on vascular and inflammatory cells [69]. Like opioids, cannabinoids that bind to CBRs have been used for centuries for medical and recreational purposes. Cannabinoids have remained controversial due to their misuse for recreational and euphoric effects [69]. Moreover, the schedule 1 status and stringent regulatory requirements have been a major deterrent in the development of these drugs for analgesia. The presence of CB1R and CB2R in the neuro-immune system makes them an attractive target for treating sickle pain. Several specific CB2R agonists have been developed to prevent the adverse effects of cannabinoids on CB1R, which is known to promote the euphoric and CNS-related effects. We found that CP55,940, a non-selective CBR agonist, which binds to both CB1R and

CB2R, ameliorates chronic and hypoxia/reoxygenation evoked hyperalgesia in sickle mice [9, 12]. However, subsequent studies targeting the contribution of individual CBRs in sickle mice show that CB1R agonists reduce mechanical, thermal and deep tissue hyperalgesia, while CB2R agonists reduce deep tissue hyperalgesia only in both chronic and acute hypoxia-/reoxygenation-evoked hyperalgesia [24]. Importantly, CB1R agonists ameliorated neurogenic inflammation, while CB2R agonists reduced mast cell activation in sickle mice, suggesting that both CB1Rs and CB2Rs are potentially critical to treat sickle pain and its underlying pathobiology.

Recently, multiple sclerosis patients experiencing spasticity and neuropathic pain exhibited significantly improved response to Sativex, a cannabis-derived oromucosal spray [70, 71]. Efficacy of Sativex for treating cancer pain is currently being tested [72], and use of cannabinoids also potentiates and improves the analgesic action of opioids in chronic pain conditions [73]. Additionally, cannabinoids attenuate ischemia/reperfusion injury [74], which is a hallmark feature of VOC in SCD.

Collectively, these results suggest that targeting CBRs may provide analgesia via not only antinociceptive mechanisms but also due to its potential to ameliorate the complex pathobiology of SCD—consequently improving the overall efficacy of the treatment. A questionnaire-based study found that 52% of the sickle patients, who self-administered marijuana, used it to relieve, reduce or prevent acute or chronic pain [75]. Therefore, CBRs offer an effective target to ameliorate pain in SCD.

5.4. Toll-like receptor 4 (TLR4)

TLR4 is the first discovered cell surface receptor of this family, which is essential for pathogen detection in innate immunity via lipopolysaccharide (LPS) recognition [76]. TLR4 has been shown to be associated with several modalities of pain including inflammatory pain [46, 77], neuropathic pain [46, 78–80], post-operative cognitive dysfunction [81], cancer pain [82], etc. Recent studies in the SCD field suggest that TLR4 activation may be a significant contributor to the multifactorial effects in SCD ranging from vaso-occlusion and inflammation to pain [83]. Heme is a product of excessive hemolysis in SCD, and heme acts as an activator for TLR4 [84]. In transgenic sickle mice, heme-activated TLR4 signaling contributes to acute lung injury (a major feature of SCD) [85] and heme-induced endothelial TLR4 activation contributes to VOC [86].

We (Gupta et al.) found that in transgenic sickle mice TLR4 expression is elevated in the spinal cord compared to control mice [12]. Spinal microglial cells are known to be involved in nociceptive signaling [46]. These cells isolated from sickle and control mice, when stimulated with hemin, exhibited activation dependent on TLR4, and this activation was mediated via ROS production and ER stress [44]. Additionally, we have observed increased expression of TLR4 in cultures of skin mast cells from sickle mice vs control mice [43]. Subsequently, genetic [87] and pharmacological [88] inhibition of TLR4 in sickle mice led to amelioration of hyperalgesia and neurogenic inflammation in transgenic sickle mice. Morphine tolerance exhibited by the SCD patients may also be a result of morphine's potential for TLR4 activation [2, 89, 90]. However, it is suggested that TLR4 may be involved in pain processing only in males [46],

whereas knocking out TLR4 affected cisplatin-induced mechanical allodynia in both male and female mice [91]. No adverse off-target effects of targeting of TLR4 in other disease conditions have been observed so far [92, 93]. Therefore, the contribution of TLR4 in sickle pain needs to be evaluated.

5.5. Other targets

A calcium-modulating serine/threonine protein kinase present in the CNS, Ca^{2+} /calmodulin protein kinase II α (CaMKII α), has been of recent interest as a modulator of neuropathic pain and is an important contributor to initiation and maintenance of opioid-induced hyperalgesia [94]. Recently, in a limited clinical trial, 18 SCD patients were treated with single dosage of trifluoperazine (a CaMKII α inhibitor) going up to 10 mg, and eight subjects reported almost 50% reduction in their chronic pain. This study established 10 mg as the toxicity limit, and the improvement in patients' health without any adverse effect warrants a randomized clinical trial to evaluate efficacy of this treatment strategy in SCD patients [95].

Dexmedetomidine, a specific α_2 -adrenoreceptor agonist, provides anti-nociception independent of opioid receptor action and via inhibition of sensory neurons [96]. This molecule also provides protection from ischemia/reperfusion injury [96]. These properties of dexmedetomidine led to a study of its efficacy in sickle mice, and Calhoun et al. found that transgenic sickle mice receiving dexmedetomidine had improved analgesia [97]. This may provide an adjuvant to existing analgesic treatment strategies used for reducing pain in SCD patients.

5.6. Integrative approaches

We observed that curcumin, an active ingredient of turmeric and Coenzyme Q10 independently ameliorated chronic hyperalgesia in sickle mice when used over a period of 4 weeks [40]. These treatments also reduced oxidative stress, microglial activation and SP in the spinal cords of sickle mice. In a clinical study on sickle patients, treatment with Coenzyme Q10 reduced the incidence of VOC [98]. In rheumatoid- and osteo-arthritis, curcumin or Theracurcumin with higher bioavailability was effective in reducing pain, inflammation and oxidative stress and symptoms of osteoarthritis in separate studies, including a randomized, double-blind, placebo-controlled trial [99, 100]. Curcumin lowered the oxidative stress and iron overload in the spleen and liver of rats with chronic iron overload [101]. Importantly, in thalassemia patients, curcumin reduced oxidative stress [102]. Thalassemia often co-exists with SCD [103], and increased iron in the tissues due to hemolysis is a characteristic feature of SCD [104]. Therefore, these dietary supplements may provide an advantage in treating sickle pathobiology and pain without the inadvertent side effects of pharmacologics discussed above.

Acupuncture has been evolving as a promising approach to relieve chronic pain. Along with several case reports [105–107], a retrospective study of 47 adult SCD patients demonstrated significant improvement in analgesia using acupuncture treatment [108]. Therefore, we developed a novel electroacupuncture (EA) method to treat awake/conscious mice to elucidate central and peripheral mechanisms contributing to acupuncture-induced analgesia without the influence of anesthesia. We found that EA in awake sickle BERK mice significantly reduces

mechanical, deep tissue and cold hyperalgesia [Wang et al., in preparation]. Response to EA was variable, but majority of sickle mice showed a high analgesic response, exhibiting reduced systemic inflammation, in addition to reduced peripheral inflammation and neuroinflammation. Integrative approaches such as acupuncture for pain control could be potentially beneficial in treating pain in SCD.

5.7. Co-treatment strategies

Mechanism-driven understanding of SCD pain pathology from basic research provides us with a variety of treatable targets as mentioned above. The promise of these different modulators of SCD pain is quite exciting; but to become viable treatment options for the SCD patients, they require systematic and rigorous clinical trials for evaluating their efficacy and any side effects that they may pose.

6. Translational potential of treatable targets-based pharmacologics

From the discussion above, it is clear that targeting sickle pain may require multiple pharmacologics due to the complex nature of SCD pathobiology and associated nociceptive mechanisms. In this regard, we can first evaluate FDA approved drugs for sickle pain based on preclinical data. Imatinib is approved by the FDA for managing chronic myeloid leukemia systemic mastocytosis [109]. Thus, mast cell inhibition via imatinib can reduce morphine-induced mast cell activation and may also enhance the efficacy of sub-optimal doses of morphine. A small study in a cohort of 17 patients using a nasal spray form of the mast cell stabilizer, cromolyn, in combination with hydroxyurea indicated that these patients experienced reduced pain when compared to placebo or to the use of cromolyn or hydroxyurea alone [110]. Thus, FDA-approved mast cell stabilizers available for reducing airway inflammation can be potentially effective as adjuvants for sickle pain.

SP acts via NK-1 receptors and NK-1 receptor antagonists have been effective in different pain pathologies in animal models, but have failed to show efficacy in clinical trials [111]. Aprepitant, an FDA-approved NK-1 receptor antagonist for chemotherapy-induced nausea and vomiting, has been assessed for the effects on electrical hyperalgesia models of human volunteers, but did not show any efficacy [112]. However, in a separate study acute doses of aprepitant were shown to significantly increase the magnitude of *mu* agonist signs and symptoms in response to oxycodone [113]. Considering the role of SP in sickle pain and neurogenic inflammation, NK-1 receptor antagonists require further examination in pre-clinical models of SCD as co-drugs.

Additionally, TLR4 inhibitors such as TAK-242 and eritoran showed promising responses in animal studies for severe sepsis, but failed to show any efficacy in reducing 28-day mortality in phase III clinical trials [114, 115]. Though these molecules are still being evaluated for other pathologic conditions such as obesity in type 2 diabetic subjects [116], no clinical trials have been undertaken using these compounds to ameliorate chronic pain conditions. Interestingly, a nonspecific phosphodiesterase (PDE4) inhibitor, ibudilast, has been shown to inhibit TLR4

and microglial activation in animal models [117] and is currently in separate clinical trials for migraine pain, multiple sclerosis and opioid abuse [118–120].

Stemming from our animal research, we are currently conducting a trial to evaluate the effect of vaporized cannabis on pain in human subjects with SCD [121]. Vaporized cannabis offers an advantage over systemically administered cannabis, because it is not metabolized by the liver and may therefore not influence organ pathology in SCD.

Apart from the targets discussed in this section, other targets such as calcium signaling and oxidative stress can be managed using pharmacologics such as trifluoperazine and curcumin/CoQ10, respectively. Curcumin and/or CoQ10 showed reduction in pain in sickle mice and CoQ10 showed reduced “crises” in a small cohort of sickle patients [40, 98]. Other integrative approaches including arginine therapy and acupuncture show reduced pain/crises in patients with SCD [108, 122]. Thus, in addition to pharmacologics, integrative approaches offer the potential to reduce sickle pain. Finally, gene therapy vectors are a new tool for the development of molecularly selective pain therapies, which have been shown to provide reliable analgesia in preclinical models [123]. The use of gene therapy may lead to a new class of analgesic treatments based on the molecular selectivity of analgesic genes.

7. Future directions

Sickle cell disease comprises highly complex pathobiology and the associated pain involves a complicated pathophysiology that we are only beginning to appreciate. Therefore, treatment strategies solely targeting the nervous system do not promise pain remission in an effective manner. Rather, as discussed in this chapter, as our understanding of the mechanistic biological targets that potentiate pain and neurogenic inflammation in SCD increases, we must incorporate multiple approaches towards alleviation of this morbid pain syndrome. The tortuous nature of SCD pain involving both central and peripheral nervous systems requires co-treatment strategies, which will ameliorate simultaneously RBC pathology leading to vaso-occlusion, mast cell activation leading to neurogenic inflammation and pain, microglial activation via increased oxidative stress, heme-induced TLR4-mediated neuronal and vascular complications, hemolysis-driven high iron/calcium-mediated pathologies, etc. Translational and clinical studies are required to evaluate the physiological relevance of these targets in order to develop effective analgesics devoid of inadvertent adverse effects. The issue of transition from acute to chronic pain is an unanswered question in SCD and other pathologies, which remains to be understood.

Acknowledgements

We thank Yann Lamarre, PhD, for the artistic illustration of **Figure 1**. Authors are also thankful to the Institute for Engineering in Medicine, University of Minnesota and NIH grants, RO1 103773 and UO1 HL117664 to K.G for funding support. The content is solely the responsibility

of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author details

Anupam Aich¹, Alvin J Beitz² and Kalpna Gupta^{1*}

*Address all correspondence to: gupta014@umn.edu

¹ Vascular Biology Center, Division of Hematology, Oncology and Transplantation, Department of Medicine, University of Minnesota, Minneapolis, MN, USA

² Department of Veterinary and Biomedical Sciences, University of Minnesota, Minneapolis, MN, USA

References

- [1] Ballas SK, Gupta K, Adams-Graves P. Sickle cell pain: a critical reappraisal. *Blood*. 2012;12018:3647–56.
- [2] Gupta M, Msambichaka L, Ballas SK, Gupta K. Morphine for the treatment of pain in sickle cell disease. *Sci World J*. 2015;2015:10.
- [3] Dampier C, Ely B, Brodecki D, Coleman C, Aertker L, Sendeki JA, et al. Pain characteristics and age-related pain trajectories in infants and young children with sickle cell disease. *Pediatr Blood Cancer*. 2014;612:291–6.
- [4] Sil S, Cohen LL, Dampier C. Psychosocial and functional outcomes in youth with chronic sickle cell pain. *Clin J Pain*. 2015;16:16.
- [5] Smith WR, Penberthy LT, Bovbjerg VE, McClish DK, Roberts JD, Dahman B, et al. Daily assessment of pain in adults with sickle cell disease. *Ann Intern Med*. 2008;1482:94–101.
- [6] Lutz B, Meiler SE, Bekker A, Tao Y-X. Updated mechanisms of sickle cell disease-associated chronic pain. *Transl Periop Pain Med*. 2015;22:8–17.
- [7] Jacob E, Chan VW, Hodge C, Zeltzer L, Zurakowski D, Sethna NF. Sensory and thermal quantitative testing in children with sickle cell disease. *J Pediatr Hematol Oncol*. 2015;373:185–9.
- [8] Brandow AM, Stucky CL, Hillery CA, Hoffmann RG, Panepinto JA. Patients with sickle cell disease have increased sensitivity to cold and heat. *Am J Hema*. 2013;881:37–43.

- [9] Cain DM, Vang D, Simone DA, Hebbel RP, Gupta K. Mouse models for studying pain in sickle disease: effects of strain, age, and acuteness. *Br J Haematol.* 2012;1564:535–44.
- [10] Garrison SR, Kramer AA, Gerges NZ, Hillery CA, Stucky CL. Sickle cell mice exhibit mechanical allodynia and enhanced responsiveness in light touch cutaneous mechanoreceptors. *Mol Pain.* 2012;862:1744–8069.
- [11] Hillery CA, Kerstein PC, Vilceanu D, Barabas ME, Retherford D, Brandow AM, et al. Transient receptor potential vanilloid 1 mediates pain in mice with severe sickle cell disease. *Blood.* 2011;11812:3376–83.
- [12] Kohli DR, Li Y, Khasabov SG, Gupta P, Kehl LJ, Ericson ME, et al. Pain-related behaviors and neurochemical alterations in mice expressing sickle hemoglobin: modulation by cannabinoids. *Blood.* 2010;1163:456–65.
- [13] Vekilov PG. Sickle-cell haemoglobin polymerization: is it the primary pathogenic event of sickle-cell anaemia? *Br J Haematol.* 2007;1392:173–84.
- [14] Kenyon N, Wang L, Spornick N, Khaibullina A, Almeida LE, Cheng Y, et al. Sickle cell disease in mice is associated with sensitization of sensory nerve fibers. *Exp Bio Med.* 2015;2401:87–98.
- [15] O'Leary JD, Crawford MW, Odame I, Shorten GD, McGrath PA. Thermal pain and sensory processing in children with sickle cell disease. *Clin J Pain.* 2014;303:244–50.
- [16] Ibrahim AS. Relationship between meteorological changes and occurrence of painful sickle cell crises in Kuwait. *Trans Royal Soc Trop Med Hyg.* 1980;742:159–61.
- [17] Redwood AM, Williams EM, Desai P, Serjeant GR. Climate and painful crisis of sickle-cell disease in Jamaica. *British Medical Journal.* 1976;16001:66–8.
- [18] Rogovik AL, Persaud J, Friedman JN, Kirby MA, Goldman RD. Pediatric vasoocclusive crisis and weather conditions. *J Emerg Med.* 2011;415:559–65.
- [19] Smith WR, Bauserman RL, Ballas SK, McCarthy WF, Steinberg MH, Swerdlow PS, et al. Climatic and geographic temporal patterns of pain in the Multicenter Study of Hydroxyurea. *Pain.* 2009;1461–2:91–8.
- [20] Nolan VG, Zhang Y, Lash T, Sebastiani P, Steinberg MH. Association between wind speed and the occurrence of sickle cell acute painful episodes: results of a case-crossover study. *Br J Haematol.* 2008;1433:433–8.
- [21] Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *Lancet.* 2010;376(9757):2018–31.
- [22] Ohara DG, Ruas G, Castro SS, Martins PR, Walsh IA. Musculoskeletal pain, profile and quality of life of individuals with sickle cell disease. *Rev Bras Fisioter.* 2012;165:431–8.

- [23] Mittal A, Gupta M, Lamarre Y, Jahagirdar B, Gupta K. Quantification of pain in sickle mice using facial expressions and body measurements. *Blood Cells Mol Dis*. 2016;57:58–66.
- [24] Vincent L, Vang D, Nguyen J, Benson B, Lei J, Gupta K. Cannabinoid receptor-specific mechanisms to ameliorate pain in sickle cell anemia via inhibition of mast cell activation and neurogenic inflammation. *Haematologica*. 2015;24:136523.
- [25] Darbari DS, Hampson JP, Ichesco E, Kadom N, Vezina G, Evangelou I, et al. Frequency of hospitalizations for pain and association with altered brain network connectivity in sickle cell disease. *J Pain*. 2015;1611:1077–86.
- [26] Mancini EA, Hillery CA, Bodian CA, Zhang ZG, Luty GA, Collier BS. Pathology of Berkeley sickle cell mice: similarities and differences with human sickle cell disease. *Blood*. 2006;1074:1651–8.
- [27] Dampier C, Ely E, Brodecki D, O'Neal P. Home management of pain in sickle cell disease: a daily diary study in children and adolescents. *J Pediatr Hematol Oncol*. 2002;248:643–7.
- [28] Brandow AM, Farley RA, Panepinto JA. Early insights into the neurobiology of pain in sickle cell disease: a systematic review of the literature. *Pediatr Blood Cancer*. 2015;629:1501–11.
- [29] Campbell CM, Moscou-Jackson G, Carroll CP, Kiley K, Haywood C, Jr., Lanzkron S, et al. An evaluation of central sensitization in patients with sickle cell disease. *J Pain*. 2016;1516:00517–4.
- [30] Ezenwa MO, Molokie RE, Wang ZJ, Yao Y, Suarez ML, Pullum C, et al. Safety and utility of quantitative sensory testing among adults with sickle cell disease: indicators of neuropathic pain? *Pain Pract*. 2015;1210:12279.
- [31] Nguyen J, Abdulla F, Chen C, Nguyen P, Nguyen M, Tittle B, et al. Phenotypic characterization the townes sickle mice. *Blood*. 2014;124(21):4916.
- [32] Fabry ME, Costantini F, Pachnis A, Suzuka SM, Bank N, Aynedjian HS, et al. High expression of human beta S- and alpha-globins in transgenic mice: erythrocyte abnormalities, organ damage, and the effect of hypoxia. *Proc Natl Acad Sci USA*. 1992;8924:12155–9.
- [33] Fabry M, Sengupta A, Suzuka S, Costantini F, Rubin E, Hofrichter J, et al. A second generation transgenic mouse model expressing both hemoglobin S (HbS) and HbS-Antilles results in increased phenotypic severity. *Blood*. 1995;866:2419–28.
- [34] Ryan TM, Ciavatta DJ, Townes TM. Knockout-transgenic mouse model of sickle cell disease. *Science*. 1997;278(5339):873–6.
- [35] Paszty C, Brion CM, Mancini E, Witkowska HE, Stevens ME, Mohandas N, et al. Transgenic knockout mice with exclusively human sickle hemoglobin and sickle cell disease. *Science*. 1997;278(5339):876–8.

- [36] Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science*. 2007;318(5858):1920–3.
- [37] Wu LC, Sun CW, Ryan TM, Pawlik KM, Ren J, Townes TM. Correction of sickle cell disease by homologous recombination in embryonic stem cells. *Blood*. 2006;1084:1183–8.
- [38] Sarray S, Saleh LR, Lisa Saldanha F, Al-Habboubi HH, Mahdi N, Almawi WY. Serum IL-6, IL-10, and TNFalpha levels in pediatric sickle cell disease patients during vaso-occlusive crisis and steady state condition. *Cytokine*. 2015;721:43–7.
- [39] Frenette PS, Atweh GF. Sickle cell disease: old discoveries, new concepts, and future promise. *J Clin Invest*. 2007;1174:850–8.
- [40] Valverde Y, Benson B, Gupta M, Gupta K. Spinal glial activation and oxidative stress are alleviated by treatment with curcumin or coenzyme Q in sickle mice. *Haematologica*. 2016;1012:e44–e7.
- [41] Beiswenger KK, Calcutt NA, Mizisin AP. Epidermal nerve fiber quantification in the assessment of diabetic neuropathy. *Acta Histochem*. 2008;1105:351–62.
- [42] Cataldo G, Rajput S, Gupta K, Simone DA. Sensitization of nociceptive spinal neurons contributes to pain in a transgenic model of sickle cell disease. *Pain*. 2015;1564:722–30.
- [43] Vincent L, Vang D, Nguyen J, Gupta M, Luk K, Ericson ME, et al. Mast cell activation contributes to sickle cell pathobiology and pain in mice. *Blood*. 2013;12211:1853–62.
- [44] Paul J, Lei J, Jha R, Nguyen J, Simone DA, Gupta K. Heme induced spinal microglial cell activation by TLR4 and endoplasmic reticulum stress in sickle mice. *Blood*. 2014;124(21):452.
- [45] Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. *Nat Rev Immunol*. 2014;144:217–31.
- [46] Sorge RE, LaCroix-Fralish ML, Tuttle AH, Sotocinal SG, Austin J-S, Ritchie J, et al. Spinal cord Toll-like receptor 4 mediates inflammatory and neuropathic hypersensitivity in male but not female mice. *J Neurosci*. 2011;3143:15450–4.
- [47] Sorge RE, Mapplebeck JCS, Rosen S, Beggs S, Taves S, Alexander JK, et al. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. *Nat Neurosci*. 2015;188:1081–3.
- [48] McClish DK, Levenson JL, Penberthy LT, Roseff SD, Bovbjerg VE, Roberts JD, et al. Gender differences in pain and healthcare utilization for adult sickle cell patients: the PiSCES project. *JWH*. 2006;152:146–54.
- [49] Wilkie DJ, Molokie R, Boyd-Seal D, Suarez ML, Kim YO, Zong S, et al. Patient-reported outcomes: descriptors of nociceptive and neuropathic pain and barriers to effective pain

- management in adult outpatients with sickle cell disease. *J Natl Med Assoc.* 2010;1021:18–27.
- [50] Brandow AM, Farley RA, Panepinto JA. Neuropathic pain in patients with sickle cell disease. *Pediatr Blood Cancer.* 2014;613:512–7.
- [51] Savage WJ, Everett AD, Casella JF. Plasma glial fibrillary acidic protein levels in a child with sickle cell disease and stroke. *Acta Haemato.* 2011;1253:103–6.
- [52] Michaels LA, Ohene-Frempong K, Zhao H, Douglas SD. Serum levels of substance P are elevated in patients with sickle cell disease and increase further during vaso-occlusive crisis. *Blood.* 1998;929:3148–51.
- [53] Li WW, Guo TZ, Liang DY, Sun Y, Kingery WS, Clark JD. Substance P signaling controls mast cell activation, degranulation, and nociceptive sensitization in a rat fracture model of complex regional pain syndrome. *Anesthesiology.* 2012;1164:882–95.
- [54] Zappia KJ, Garrison SR, Hillery CA, Stucky CL. Cold hypersensitivity increases with age in mice with sickle cell disease. *Pain.* 2014;15512:2476–85.
- [55] Werner MF, Trevisani M, Campi B, Andre E, Geppetti P, Rae GA. Contribution of peripheral endothelin ETA and ETB receptors in neuropathic pain induced by spinal nerve ligation in rats. *Eur J Pain.* 2010;149:911–7.
- [56] Teodoro FC, Tronco Júnior MF, Zampronio AR, Martini AC, Rae GA, Chichorro JG. Peripheral substance P and neurokinin-1 receptors have a role in inflammatory and neuropathic orofacial pain models. *Neuropeptides.* 2013;473:199–206.
- [57] Smith M, Wally R, McClish P, Donna K, Dahman P, Bassam A, Levenson M, James L, Aisiku M, Imoigele P, Citero M, Vanessa de A, et al. Daily home opioid use in adults with sickle cell disease: the PiSCES project. *J Opioid Manag.* 2015;113:11.
- [58] Chu LF, Angst MS, Clark D. Opioid-induced hyperalgesia in humans: molecular mechanisms and clinical considerations. *Clin J Pain.* 2008;246:479–96.
- [59] Weber ML, Vang D, Velho PE, Gupta P, Crosson JT, Hebbel RP, et al. Morphine promotes renal pathology in sickle mice. *Int J Nephrol Renovasc Dis.* 2012;5:109–18.
- [60] Wang X, Loram LC, Ramos K, de Jesus AJ, Thomas J, Cheng K, et al. Morphine activates neuroinflammation in a manner parallel to endotoxin. *Proc Natl Acad Sci USA.* 2012;10916:6325–30.
- [61] Chen C, Farooqui M, Gupta K. Morphine stimulates vascular endothelial growth factor-like signaling in mouse retinal endothelial cells. *Curr Neurovasc Res.* 2006;33:171–80.
- [62] Wang Y, Barker K, Shi S, Diaz M, Mo B, Gutstein HB. Blockade of PDGFR-[beta] activation eliminates morphine analgesic tolerance. *Nat Med.* 2012;183:385–7. DOI: 10.1038/nm.2633

- [63] Zaveri NT. The nociceptin/orphanin FQ receptor (NOP) as a target for drug abuse medications. *Curr Top Med Chem*. 2011;119:1151–6.
- [64] Helyes Z, Nemeth J, Pinter E, Szolcsanyi J. Inhibition by nociceptin of neurogenic inflammation and the release of SP and CGRP from sensory nerve terminals. *Br J Pharmacol*. 1997;1214:613–5.
- [65] Nemeth J, Helyes Z, Oroszi G, Than M, Pinter E, Szolcsanyi J. Inhibition of nociceptin on sensory neuropeptide release and mast cell-mediated plasma extravasation in rats. *Eur J Pharmacol*. 1998;3471:101–4.
- [66] Vang D, Paul JA, Nguyen J, Tran H, Vincent L, Yasuda D, et al. Small-molecule nociceptin receptor agonist ameliorates mast cell activation and pain in sickle mice. *Haematologica*. 2015;10012:1517–25.
- [67] Aich A, Afrin L, Gupta K. Mast cell-mediated mechanisms of nociception. *Intl J Mol Sci*. 2015;1612:26151.
- [68] Inceoglu B, Bettaieb A, Trindade da Silva CA, Lee KSS, Haj FG, Hammock BD. Endoplasmic reticulum stress in the peripheral nervous system is a significant driver of neuropathic pain. *Proc Natl Acad Sci USA*. 2015;112(29):9082–7.
- [69] Gupta M, Thompson S, Gupta K, Abrams DI. Cannabis in the treatment of pain in sickle cell disease. *JSCDH*. 2016. In press.
- [70] Collin C, Ehler E, Waberzinek G, Alsindi Z, Davies P, Powell K, et al. A double-blind, randomized, placebo-controlled, parallel-group study of Sativex, in subjects with symptoms of spasticity due to multiple sclerosis. *Neurol Res*. 2010;325:451–9.
- [71] Barnes MP. Sativex: clinical efficacy and tolerability in the treatment of symptoms of multiple sclerosis and neuropathic pain. *Expert Opin Pharmacother*. 2006;75:607–15.
- [72] Brower V. New pain drugs in pipeline, but challenges to usage remain. *J Nat Can Inst*. 2012;1047:503–5.
- [73] Abrams DI, Couey P, Shade SB, Kelly ME, Benowitz NL. Cannabinoid-opioid interaction in chronic pain. *Clin Pharmacol Ther*. 2011;906:844–51.
- [74] Zhang M, Adler MW, Abood ME, Ganea D, Jallo J, Tuma RF. CB2 receptor activation attenuates microcirculatory dysfunction during cerebral ischemic/reperfusion injury. *Microvasc Res*. 2009;781:86–94.
- [75] Howard J, Anie KA, Holdcroft A, Korn S, Davies SC. Cannabis use in sickle cell disease: a questionnaire study. *Br J Haematol*. 2005;1311:123–8.
- [76] Takeda K, Akira S. Toll-like receptors in innate immunity. *Intl Immun*. 2005;171:1–14.
- [77] Qian B, Li F, Zhao L-X, Dong Y-L, Gao Y-J, Zhang Z-J. Ligustilide ameliorates inflammatory pain and inhibits TLR4 upregulation in spinal astrocytes following complete Freund's adjuvant peripheral injection. *Cell Mol Neurob*. 2015;361:143–9.

- [78] Bettoni I, Comelli F, Rossini C, Granucci F, Giagnoni G, Peri F, et al. Glial TLR4 receptor as new target to treat neuropathic pain: efficacy of a new receptor antagonist in a model of peripheral nerve injury in mice. *Glia*. 2008;5612:1312–9.
- [79] Jurga AM, Rojewska E, Piotrowska A, Makuch W, Pilat D, Przewlocka B, et al. Blockade of toll-like receptors (TLR2, TLR4) attenuates pain and potentiates buprenorphine analgesia in a rat neuropathic pain model. *Neural Plasticity*. 2016;2016:12.
- [80] Wu F-x, Bian J-j, Miao X-r, Huang S-d, Xu X-w, Gong D-j, et al. Intrathecal siRNA against toll-like receptor 4 reduces nociception in a rat model of neuropathic pain. *Intl J Med Sci*. 2010;75:251–9.
- [81] Lu S-M, Yu C-J, Liu Y-H, Dong H-Q, Zhang X, Zhang S-S, et al. S100A8 contributes to postoperative cognitive dysfunction in mice undergoing tibial fracture surgery by activating the TLR4/MyD88 pathway. *Brain Behav Immun*. 2015;44:221–34.
- [82] Lan LS, Ping YJ, Na WL, Miao J, Cheng QQ, Ni MZ, et al. Down-regulation of Toll-like receptor 4 gene expression by short interfering RNA attenuates bone cancer pain in a rat model. *Mol Pain*. 2010;61:1–13.
- [83] Gupta K. HMGB1 takes a “Toll” in sickle cell disease. *Blood*. 2014;124(26):3837–8.
- [84] Figueiredo RT, Fernandez PL, Mourao-Sa DS, Porto BN, Dutra FF, Alves LS, et al. Characterization of heme as activator of Toll-like receptor 4. *J Biol Chem*. 2007;282(28):20221–9.
- [85] Ghosh S, Adisa OA, Chappa P, Tan F, Jackson KA, Archer DR, et al. Extracellular hemin crisis triggers acute chest syndrome in sickle mice. *J Clin Invest*. 2013;123(11):4809–20.
- [86] Belcher JD, Chen C, Nguyen J, Milbauer L, Abdulla F, Alayash AI, et al. Heme triggers TLR4 signaling leading to endothelial cell activation and vaso-occlusion in murine sickle cell disease. *Blood*. 2014;123(3):377–90.
- [87] Vang D, Pena RDS, Robiner SA, Gupta K. Toll-like receptor 4 knockout attenuates neurogenic inflammation and hyperalgesia in sickle mice. *Blood*. 2013;122(21):732.
- [88] Lei J, Wang Y, Paul J, Thompson S, Jha R, Gupta K, editors. Pharmacological inhibition of TLR4 reduces mast cell activation, neuroinflammation and hyperalgesia in sickle mice. *ASH 57th Annual Meeting & Exposition, Orlando, Florida; 2015*.
- [89] Grace PM, Strand KA, Galer EL, Zhang Y, Berkelhammer D, Greene LI, et al. Therapeutic morphine prolongs neuropathic pain in rats: a role for TLR4 and inflammasome signaling in the lumbar spinal cord. *Brain Behav Imm*. 2014;40(Suppl.):e9–e10.
- [90] Hutchinson MR, Shavit Y, Grace PM, Rice KC, Maier SF, Watkins LR. Exploring the neuroimmunopharmacology of opioids: an integrative review of mechanisms of central immune signaling and their implications for opioid analgesia. *Pharmacol Rev*. 2011;633:772–810.

- [91] Woller SA, Corr M, Yaksh TL. Differences in cisplatin-induced mechanical allodynia in male and female mice. *Eur J Pain*. 2015;1910:1476–85.
- [92] Hutchinson MR, Loram LC, Zhang Y, Shridhar M, Rezvani N, Berkelhammer D, et al. Evidence that tricyclic small molecules may possess toll-like receptor and MD-2 activity. *Neuroscience*. 2010;1682:551–63.
- [93] Savva A, Roger T. Targeting toll-like receptors: promising therapeutic strategies for the management of sepsis-associated pathology and infectious diseases. *Front Immun*. 2013;4:4 387–N/A.
- [94] Chen Y, Yang C, Wang ZJ. Ca²⁺/calmodulin-dependent protein kinase II α is required for the initiation and maintenance of opioid-induced hyperalgesia. *J Neurosci*. 2010;301:38–46.
- [95] Molokie RE, Wilkie DJ, Wittert H, Suarez ML, Yao Y, Zhao Z, et al. Mechanism-driven phase I translational study of trifluoperazine in adults with sickle cell disease. *Eur J Pharmacol*. 2014;723:419–24.
- [96] Yoshitomi O, Cho S, Hara T, Shibata I, Maekawa T, Ureshino H, et al. Direct protective effects of dexmedetomidine against myocardial ischemia-reperfusion injury in anesthetized pigs. *Shock*. 2012;381:92–7.
- [97] Calhoun G, Wang L, Almeida LEF, Kenyon N, Afsar N, Nouraie M, et al. Dexmedetomidine ameliorates nocifensive behavior in humanized sickle cell mice. *Eur J Pharmacol*. 2015;754:125–33.
- [98] Thakur AS, Littaru GP, Moesgaard S, Dan Sindberg C, Khan Y, Singh CM. Hematological parameters and RBC TBARS level of Q 10 supplemented tribal sickle cell patients: a hospital based study. *Indian J Clin Biochem*. 2013;282:185–8.
- [99] Gupta SC, Patchva S, Aggarwal BB. Therapeutic roles of curcumin: lessons learned from clinical trials. *Aaps J*. 2013;151:195–218.
- [100] Nakagawa Y, Mukai S, Yamada S, Matsuoka M, Tarumi E, Hashimoto T, et al. Short-term effects of highly-bioavailable curcumin for treating knee osteoarthritis: a randomized, double-blind, placebo-controlled prospective study. *J Orthop Sci*. 2014;196:933–9.
- [101] Badria FA, Ibrahim AS, Badria AF, Elmarakby AA. Curcumin attenuates iron accumulation and oxidative stress in the liver and spleen of chronic iron-overloaded rats. *PLoS One*. 2015;107:e0134156–N/A.
- [102] Kalpravidh RW, Siritanaratkul N, Insain P, Charoensakdi R, Panichkul N, Hatairaktham S, et al. Improvement in oxidative stress and antioxidant parameters in beta-thalassemia/Hb E patients treated with curcuminoids. *Clin Biochem*. 2010;434–5:424–9.
- [103] King A, Shenoy S. Evidence-based focused review of the status of hematopoietic stem cell transplantation as treatment of sickle cell disease and thalassemia. *Blood*. 2014;12320:3089–94.

- [104] Raghupathy R, Manwani D, Little JA. Iron overload in sickle cell disease. *Adv Hema*. 2010;2010:272940–N/A.
- [105] Marques CVP. Laser acupuncture to manage pain in child with sickle cell disease. Case report. *Relato de caso. Revista Dor*. 2014;15:70–3.
- [106] Tsai S-L, McDaniel D, Taromina K, Lee MT. Acupuncture for sickle cell pain management in a pediatric emergency department, hematology clinic, and inpatient unit. *Med Accup*. 2015;276:510–4.
- [107] Bhushan D, Conner K, Ellen JM, Sibinga EMS. Adjuvant acupuncture for youth with sickle cell pain: a proof of concept study. *Med Accup*. 2015;276:461–6.
- [108] Lu K, Cheng MC, Ge X, Berger A, Xu D, Kato GJ, et al. A retrospective review of acupuncture use for the treatment of pain in sickle cell disease patients: descriptive analysis from a single institution. *Clin J Pain*. 2014;309:825–30.
- [109] Iqbal N, Iqbal N. Imatinib: A breakthrough of targeted therapy in cancer. *Chemother Res Pract*. 2014;2014:9.
- [110] Karimi M, Zekavat OR, Sharifzadeh S, Mousavizadeh K. Clinical response of patients with sickle cell anemia to cromolyn sodium nasal spray. *Am J Hematol*. 2006;8111:809–16.
- [111] Garcia-Recio S, Gascón P. Biological and pharmacological aspects of the NK1-receptor. *BioMed Res Inter*. 2015;2015:14.
- [112] Chizh BA, Gohring M, Troster A, Quartey GK, Schmelz M, Koppert W. Effects of oral pregabalin and aprepitant on pain and central sensitization in the electrical hyperalgesia model in human volunteers. *Br J Anaesth*. 2007;982:246–54.
- [113] Walsh SL, Heilig M, Nuzzo PA, Henderson P, Lofwall MR. Effects of the NK1 antagonist, aprepitant, on response to oral and intranasal oxycodone in prescription opioid abusers. *Addict Biol*. 2013;182:332–43.
- [114] Opal SM, Laterre PF, Francois B, LaRosa SP, Angus DC, Mira JP, et al. Effect of eritoran, an antagonist of MD2-TLR4, on mortality in patients with severe sepsis: the ACCESS randomized trial. *JAMA*. 2013;30911:1154–62.
- [115] Rice TW, Wheeler AP, Bernard GR, Vincent JL, Angus DC, Aikawa N, et al. A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis. *Crit Care Med*. 2010;388:1685–94.
- [116] Musi N. The effect of TLR4 inhibition in obese and type 2 diabetic subjects (Eritoran 2). Available from: <https://clinicaltrials.gov/ct2/show/NCT02267317> [cited 2016 April 19].
- [117] Zhaleh M, Panahi M, Ghafurian Broujerdnia M, Ghorbani R, Ahmadi Angali K, Saki G. Role of phosphodiesterase inhibitor Ibudilast in morphine-induced hippocampal injury. *J Inj Violence Res*. 2014;62:72–8.

- [118] Rolan P, Gazerani P. Ibutilast in the treatment of patients with chronic migraine (IBU-003). Available from: <https://clinicaltrials.gov/ct2/show/NCT01389193?term=ibutilast&rank=2> [cited 2016 April 19].
- [119] Fox RJ. Safety, tolerability and activity study of ibutilast in subjects with progressive multiple sclerosis. Available from: <https://clinicaltrials.gov/ct2/show/NCT01982942?term=ibutilast&rank=7> [cited 2016 April 19].
- [120] Comer SD. Effects of ibutilast on oxycodone self-administration in Opioid Abusers. Available from: <https://clinicaltrials.gov/ct2/show/NCT01740414?term=ibutilast&rank=8> [cited 2016 April 19].
- [121] Abrams DI. Vaporized cannabis for chronic pain associated with sickle cell disease (Cannabis-SCD). ClinicalTrials.gov. Available from: <https://clinicaltrials.gov/ct2/show/NCT01771731?term=sickle+pain&rank=40> [cited 2016 February 27].
- [122] Morris CR, Kuypers FA, Lavrisha L, Ansari M, Sweeters N, Stewart M, et al. A randomized, placebo-controlled trial of arginine therapy for the treatment of children with sickle cell disease hospitalized with vaso-occlusive pain episodes. *Haematologica*. 2013;989:1375–82.
- [123] Pleticha J, Maus TP, Beutler AS. Future directions in pain management. *Mayo Clin Proc*. 2016;914:522–33.