Pain mechanisms in sickle cell disease. Are we closer to a breakthrough?

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In this issue of *Haematologica*, Khasabova *et al.*[1] demonstrate a role for accelerated biosynthesis of the endocannabinoid, 2-arachidonoylglycerol (2-AG), and thereby prostaglandin E_2-glycerol (PGE_2-G) generation, in the hyperalgesia observed in a murine model of sickle cell disease (SCD). Pain is a hallmark of SCD and is a major cause of morbidity in patients, with significant negative effects on quality of life. Acute pain, a characteristic and frequent complication of SCD, is usually generated by vaso-occlusive episodes,[2] in which vaso-occlusion and ensuing ischemia-reperfusion processes generate the production of multiple pro-inflammatory molecules and pain mediators, including eicosanoids and bradykinin.[3,4] The causes of chronic pain, previously reported to affect approximately 30% of adults with SCD on an almost daily basis,[5] are less clear in SCD, but may arise from central sensitization due to nociceptive signaling from the periphery to the central nervous system, leading to pain hypersensitivity, although there is also evidence for a contribution of neuropathic pain.[4]

Following on from their previous study[6] showing that sensitization of nociceptors by PGE_2-G in mice with SCD contributes to hyperalgesia (defined as an increased sensitivity to pain), Khasabova *et al.*[1] now go on to show that the majority, but not all, of SCD mice (HbSS Berkeley model) studied exhibit strong mechanical and heat hyperalgesia and that this hyperalgesia is associated with significantly higher plasma levels of 2-AG, as compared to mice without SCD (HbAA) and to SCD mice that are not hyperalgesic. Endocannabinoids, such as 2-AG, are endogenous bioactive lipids that have been proposed as novel therapeutic targets for modulating inflammatory nociceptive pain. 2-AG is often regarded as anti-nociceptive upon its binding to cannabinoid receptors, but becomes pro-nociceptive when metabolized by cyclooxygenase-2 (COX-2) to PGE_2-G, and may play a key role in the transformation of acute pain to chronic pain.[7]

Consistent with a proposed role for increased 2-AG endocannabinoid in the hyperalgesia observed in SCD mice, the administration of exogenous 2-AG to non-hyperalgesic HbSS mice, but not to HbAA mice, induced rapid mechanical hyperalgesia that persisted for 24 hours. Inhibition of 2-AG hydrolysis, to elevate endogenous 2-AG concentrations, also generated hyperalgesia in non-hyperalgesic hemizygous HbAS mice as well as in HbSS mice. While higher plasma 2-AG levels were limited to the population of HbSS mice that showed hyperalgesia, COX-2 protein (which oxygenates 2-AG to generate PGE_2-G) was elevated in the blood cells of all HbSS mice, regardless of their hyperalgesic classification, and higher than in HbAA mice. This may explain why hyperalgesia can be induced by 2-AG in non-hyperalgesic HbSS mice, but not in HbAA mice. Addressing the question of whether the elevated 2-AG levels in HbSS mice were due to increased biosynthesis or decreased hydrolysis, the authors found that hyperalgesia in these mice was associated with an increased peripheral blood cell content of diacylglycerol lipase-β (DAGLβ), an enzyme that synthesizes 2-AG from diacylglycerides. Consistent with the hypothesis that elevated DAGLβ expression or activity may accelerate 2-AG biosynthesis and induce the PGE_2-G-mediated hyperalgesia observed in SCD mice, administration of a selective inhibitor of DAGLβ temporarily reduced mechanical and heat hyperalgesia in HbSS mice and also decreased circulating concentrations of 2-AG, PGE_2 and PGE_2-G in mice with SCD.

The management of pain, both acute and chronic, in SCD often requires the use of opioids for analgesia, but challenges can arise from the side-effects associated with such medications, opioid-induced hyperalgesia and, sadly, some provider bias.[2] As such, the search continues to identify effective non-opioid-based analgesic therapeutic approaches for pain in SCD. The use of COX-2 inhibitors has previously been suggested for managing chronic pain in SCD,[2,8] especially given evidence of elevated COX-2 expression and/or activity in the leukocytes of mice and patients with SCD,[1,6,9] with Khasabova *et al.* previously reporting on the analgesic efficacy of *R*-flurbiprofen in mice with SCD.[6] However, observations in the latest study by Khasabova and colleagues indicate that elevation of 2-AG, upstream of COX-2, may be specific to those SCD mice

displaying hyperalgesia,[1] meaning that approaches that can decrease DAGLβ-mediated biosynthesis of 2-AG could provide targeted relief for hyperalgesia in SCD, with theoretically fewer side effects than those of COX inhibitors.[10] Furthermore, combined administration of a selective DAGLβ inhibitor together with opioids could potentially lower the dose of opioid required for analgesia of SCD pain.[1,2]

One intriguing point of interest that arises from the study by Khasabova *et al.*[1] is that not all the transgenic HbSS mice studied displayed hyperalgesia, and that the hyperalgesia observed arose from a peripheral mechanism of pain. DAGL expression occurs differentially, with DAGLα expression restricted essentially to the central nervous system and DAGLβ activity occurring in immune cells, particularly macrophages. How DAGLβ protein expression is upregulated in the cellular component of the peripheral blood of hyperalgesic HbSS mice, but not in non-hyperalgesic HbSS mice, was not explored, but alterations in the immune cell profile of these mice are possible, and pancellular leukocyte activation is also a characteristic of SCD. Immunoreactivity for DGLβ has been associated with tumor necrosis factor-α expression in $CD68^+$ monocytes/macrophages in a murine model of inflammatory pain; importantly, selective inhibition of the DAGLβ enzyme, or knockout of its gene, was also shown to prevent pro-inflammatory responses in mouse peritoneal macrophages and allodynic pain responses in the lipopolysaccharide model of inflammatory pain in mice.[10] Thus, taken together, a contribution of inflammatory processes to DGLβ upregulation in SCD mice, and hence to the hyperalgesia observed in a subset of these mice, may be suggested. Understanding pain, in the context of the complex pathophysiology of SCD, is a daunting task, but observations such as those reported in this study may throw some light onto the role that peripheral mechanisms of inflammatory pain may play in the progression of acute pain to chronic pain in SCD, and the pain hypersensitivity that can occur in the disease.

Disclosures

No conflicts of interest to disclose.

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