VIEWPOINT

Targeting neuroinflammation in neuropathic pain and opioid use

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Neuropathic pain arises from injuries to the nervous system. It affects 20% of the adult US population and poses a major socioeconomic burden yet remains exceedingly difficult to treat. Current therapeutic approaches have limited efficacy and a large side effect profile that impedes their ability to treat neuropathic pain effectively. Preclinical research over the last 30 yr has established the critical role that pro-inflammatory neuro–immune cell interactions have in the development and maintenance of neuropathic pain arising from various etiologies. Pro-inflammatory neuro–immune cell interactions also underlie the development of adverse side effects of opioids and the loss of their efficacy to treat pain. Evidence from work in our lab and others in preclinical animal models have shown that signaling from the bioactive sphingolipid, sphingosine-1-phosphate (S1P), through the S1P receptor subtype 1 (S1PR1) modulates neuro–immune cell interactions. Here, we discuss how targeting S1P/S1PR1 signaling with S1PR1 antagonists already Food and Drug Administration–approved or in clinical trials for multiple sclerosis can provide a viable pharmacotherapeutic approach to reduce neuro-immune cell inflammatory signaling and potentially treat patients suffering neuropathic pain and the adverse effects of opioids.

Neuropathic pain affects about 20% of the adult US population and poses a major socioeconomic burden (Dahlhamer et al., 2018). Neuropathic pain arises from injuries to the nervous system due to trauma, disease, or exposure to neurotoxins (e.g., chemotherapeutics). The nerve injury results in chronic changes to primary afferent sensory neurons that enhance their sensitivity and excitability to noxious and non-noxious stimuli (peripheral sensitization). Nerve injury also elicits chronic changes in the central nervous system (CNS) that enhance the excitability of those neurons that respond to and process sensory peripheral input (central sensitization; Colloca et al., 2017).

Neuropathic pain is exceedingly difficult to treat. The few current pharmacotherapeutic options, such as antidepressants, anticonvulsants, and opioids, have limited efficacy and many side effects, as exemplified by the "opioid epidemic" (Colloca et al., 2017). These drugs blunt neuropathic pain by directly targeting neurons to decrease their excitability and neurotransmission

(Colloca et al., 2017). However, immune and inflammatory signaling interactions between neurons and glia (astrocytes and microglia) in the CNS or lymphocytes and macrophages that infiltrate the dorsal root ganglia and spinal cord in response to injury play key roles in the development of enhanced neuronal excitability and central sensitization associated with neuropathic pain states (Finnerup et al., 2021). Such neuro–immune interactions increase neuronal excitability through many mechanisms. For example, pro-nociceptive mediators (e.g., glial-derived adenosine triphosphate and glutamate) and inflammatory cytokines (e.g., TNF and IL-1β) that enhance excitatory neurotransmission and modulate neuron ion channel conduction are released by immune cells in the periphery and by activated neuroinflammatory glia in the CNS following nerve injury (Ji et al., 2016). IL-1β can affect neuronal excitatory synaptic transmission through phosphorylation of postsynaptic NMDA receptor subunits, the downregulation of key astrocyte-restricted glutamate transporters

that account for >80% of glutamate reuptake at the excitatory synapse, and the downregulation of neuronal GPCR kinase 2 (GRK2; an enzymatic regulator of the homologous desensitization of many GPCRs that protects against over-stimulation; Grace et al., 2014). Cytokines also decrease glutamine synthetase in astrocytes, an enzyme which is responsible for converting excess glutamate into nontoxic glutamine (Grace et al., 2014). These effects are amplified by increased formation of reactive oxygen and nitrogen species, such as superoxide and peroxynitrite, that contribute to persistent glutamatergic signaling by inactivating glutamate transporters and glutamine synthetase (Squillace and Salvemini, 2022). Illustrating the critical role of neuroinflammation to neuropathic pain, gene therapy strategies that enhance endogenous IL-4 and IL-10 anti-inflammatory signaling provide sustained suppression of neuroinflammation and improve chronic pain condition in animal models (Vanderwall and Milligan, 2019).

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Abundant data over the years have also implicated important roles for neuro–immune interactions in the development of side effects associated with analgesics that are used to treat neuropathic pain (Roeckel et al., 2016). For example, sustained administration of morphine can lead to the loss of opioid efficacy (analgesic tolerance), extend the duration of neuropathic pain (opioid-induced persistence of pain), and itself produce paradoxical pain hypersensitivity (opioid-induced hyperalgesia [OIH]; Roeckel et al., 2016). These effects are associated with activated glial cells, reduced levels of astrocyte glutamate transporter activity, elevated levels of glial-derived inflammatory cytokines (e.g., TNF, IL-1β) and chemokines (e.g., CCL5, IL-8), and excessive production of reactive oxygen and nitrogen species within the spinal cord (Grace et al., 2015; Salvemini and Neumann, 2009). Neuro–immune inhibition with cytokine antagonists (e.g., IL-1 receptor antagonist or soluble TNF receptor), toll-like receptor antagonists, and immunomodulatory drugs (e.g., propentofylline) have reduced opioid tolerance and OIH in animal models (Roeckel et al., 2016). Likewise, inhibiting superoxide/peroxynitrite signaling blocks OIH and opioid tolerance (Salvemini and Neumann, 2009).

Collectively, such data suggest that targeting neuronal–immune cell interactions represent an exciting avenue towards the development of novel non-narcotic analgesics. In these settings, a pressing question is whether we can identify specific cellular targets within a complex network of pathways involved in neuro–immune cell interactions to attenuate central sensitization and effectively manage neuropathic pain states. Our work and that of others suggests that this goal can be achieved by targeting the sphingosine-1-phosphate (S1P) receptor subtype 1 (S1PR1; Squillace et al., 2020). Numerous studies reveal that traumatic nerve injuries, chemotherapy, autoimmune disorders, and sustained opioid use alter sphingolipid metabolism in the CNS and increase the production of ceramide and its downstream metabolite S1P, a potent signaling molecule (Singh and Spiegel, 2020). Once formed, S1P is released from cells to initiate autocrine and paracrine signaling by activating five known G protein–coupled S1P receptor subtypes (S1PR1-5; Singh and Spiegel, 2020). Our

work and that of others implicates S1PR1 in the development of neuropathic pain states, OIH, and opioid analgesic tolerance (Squillace et al., 2020). S1PR1 signaling has long been recognized to play a role in inflammation, such as lymphocyte and macrophage chemotaxis and the modulation of inflammatory/anti-inflammatory macrophage phenotypes (Singh and Spiegel, 2020). In multiple sclerosis, blocking S1PR1 signaling on lymphocytes impairs T cell release from lymphoid tissues, reducing their infiltration into the CNS and impeding neuro-inflammation and the progression of neurodegeneration (Brinkmann, 2009). In the brain, S1PR1 is highly expressed in astrocytes relative to microglia and neurons (Squillace et al., 2020), and the loss of S1PR1 in astrocytes in animal models of multiple sclerosis reduces inflammatory CNS cytokine levels and slows the progression of neurodegeneration (Choi et al., 2011). Our work revealed that S1PR1 in astrocytes also contributes to the development of neuropathic pain and OIH (Squillace et al., 2020). We found that injecting the S1PR1 agonist SEW2871 into the spinal cord of uninjured animals was sufficient to produce pain hypersensitivity. This effect involved activating astrocyte-specific S1PR1 in the spinal cord and stimulating IL-1β production and its required post-translational processing by the nod-like receptor family pyrin domain containing protein 3 (NLRP3) inflammasome (Doyle et al., 2020). Pharmacological disruption of this S1PR1-mediated and NLRP3-mediated increase in IL-1β signaling resulted in a compensatory increase in IL-10 signaling that attenuated hypersensitivity (Doyle et al., 2020). IL-1β signaling in the spinal cord also activates sphingosine kinase activity to produce S1P. Inhibition of S1P production or S1PR1 attenuated IL-1β–induced mechanical hypersensitivity in rats (Doyle et al., 2020).

Over the last decade, several orally bioavailable and CNS-penetrant S1PR1 functional and competitive antagonists have been developed (Squillace et al., 2020). S1PR1 functional antagonists are compounds that bind to S1PR1 as an agonist but deplete S1PR1 expression on the cell membrane by preventing the recycling of internalized S1PR1 back to the cell surface (Brinkmann, 2009). Two S1PR1 functional antagonists are now Food and Drug Administration–approved for the treatment of multiple sclerosis: the

pro-drug FTY720 (fingolimod; Gilenya, Novartis), approved in 2010, and ozanimod (RPC1063, Zeposia, Celgene), approved in 2020 (Squillace et al., 2020). S1PR1 competitive antagonists such as NIBR-15 and TASP0277308 are in advanced preclinical development for a variety of disease states (Squillace et al., 2020). Multiple functional and competitive S1PR1 antagonists have been shown to be beneficial in preventing and reversing neuropathic pain in several animal models at doses below those required for immunosuppression (Squillace et al., 2020). Moreover, selective S1PR1 agonists such as SEW2871 do not block neuropathic pain and in some cases may even exacerbate it (Squillace et al., 2020). In contrast, a study in experimental autoimmune encephalomyelitis (EAE; an animal model of multiple sclerosis) concluded that activation of S1PR1, not its inhibition, is responsible for the antinociceptive effects of FTY720 in this model since similar effects were obtained with an agonist and antagonist (Doolen et al., 2018). Here, in the EAE model, beneficial effects obtained with systemic administration of the functional S1PR1 antagonist, FTY720, were mimicked by a selective S1PR1 agonist, SEW2871, and both approaches reduced hyperalgesia (Doolen et al., 2018). However, S1PR1 agonists and functional S1PR1 antagonists reduce EAE disease progression in part by reducing lymphocyte migration and their access to the CNS (Brinkmann, 2009). Decreased infiltration of reactive immune cells in the CNS is anticipated to attenuate neuro–immune signaling in the CNS, decreasing central sensitization. Additional work targeting S1PR1 is needed in models of autoimmune-driven neuropathic pain states.

In addition to their effects in models of neuropathic pain states, S1PR1 antagonists attenuate important adverse effects of medications such as opioids used in the treatment of neuropathic pain. Sustained morphine treatment in rodents activate the metabolic machinery involved in the production of S1P and S1PR1 signaling in the CNS that leads to OHI and analgesic tolerance (Squillace et al., 2020). In models of neuropathic pain and OIH/analgesic tolerance, S1PR1 antagonists attenuate glial activation, NLRP3 signaling, and inflammatory cytokine production (e.g., TNF, IL-1β, and IL-6) in the spinal cord of animals with neuropathic pain or OIH (Squillace et al., 2020). Moreover, these

antagonists lose their effects if S1PR1 is deleted from astrocytes, suggesting that these cells are a prime pharmacological target (Squillace et al., 2020). Based on data from our studies that show that the anti-hypersensitivity effects of S1PR1 antagonists are lost by inhibiting anti-inflammatory IL-10 signaling (Squillace et al., 2020) and studies in glia from mouse models of multiple sclerosis (Rothhammer et al., 2017), we now think the underlying mechanisms of action for S1PR1 antagonists include an ability to induce a change in glia from the inflammatory to the anti-inflammatory phenotype.

In summary, preclinical studies provide support for S1PR1 as a viable target for therapeutic intervention with S1PR1 antagonists. S1PR1 antagonists interfere with neuro–immune signaling through multiple mechanisms; such polypharmacological activity is likely responsible for the efficacy and potency seen with S1PR1 antagonists in multiple preclinical pain models. Moreover, emerging evidence suggests that targeting S1PR1 may impact important comorbidities associated with neuropathic pain states such as cognitive impairment by preventing neuro–immune signaling in the CNS (Squillace et al., 2022). There is now a strong need to explore repurposing drugs such as FTY720 and ozanimod and developing new S1PR1 antagonists for the treatment of pain of diverse etiologies. We think it is very likely that as clinical studies progress and more is revealed about the mechanisms of S1P and S1PR1 in neuropathic pain and opioid-induced adverse effects, the S1P signaling pathway will be a target of growing interest for the development of new approaches to combat pain.

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