A Safe Strategy for Control of Hyperalgesia - Boswellic Acids, Phycocyanobilin, and Salsalate

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Abstract

Hyperalgesia often develops in response to inflammation, peripheral nerve damage, and neurotoxic cancer chemotherapies. Although NSAIDs and opiates can usually suppress pain in these circumstances, increased risks for GI bleeding, renal damage, and vascular events, and the propensity of chronic opiate therapy to give rise to tolerance and even hyperalgesia, render these measures far from optimal for long-term use. The development of hyperalgesia is typically associated with, and in part driven by, oxidative stress, NF-kappaB activation, and increased PGE2 synthesis reflecting cox-2 induction, both in primary sensory afferents and in the spinal dorsal horn where the cell bodies of secondary afferents are located. It may therefore prove feasible to safely control hyperalgesia with a regimen comprised of: the spirulina chromophore phycocyanobilin – which has the potential to inhibit NADPH oxidase-mediated superoxide production in primary afferents and in microglia within the spinal dorsal horn; salsalate – which can suppress NF-kappaB activation via inhibition of IkappaB kinase-beta; and beta-boswellic acid – which has recently emerged as clinically useful inhibitor of microsomal prostaglandin E synthase-1, the chief source of PGE2 when cox-2 is induced. N-acetylcysteine and melatonin could also be employed to complement the antioxidant efficacy of phycocyanobilin. Each of these agents, as contrasted to NSAIDs or opiates, appears to be well-tolerated, aside from the dose-limiting reversible ototoxicity evoked by salsalate. These agents merit evaluation in rodent models of hyperalgesia, and if results are promising, the clinical impact of the proposed regimen could be assessed.

Current Therapies for Hyperalgesia Have Drawbacks

Although efficient transmission of acute nociceptive stimuli is essential to avoidance of injury, there are many circumstances in which primary sensory afferents, the second-order spinal sensory neurons arising in the dorsal horn, as well as supraspinal pain centers, become supersensitized to activating signals, such that trivial peripheral stimulation can give rise to obnoxious pain perception; this phenomenon is known as hyperalgesia. Hyperalgesia can arise in response to chronic peripheral inflammation, injury to peripheral neurons (neuropathic pain), administration of certain neurotoxic chemotherapy drugs, and – perversely - chronic opiate therapy; it is also a characteristic feature of certain poorly understood disorders such as fibromyalgia and chronic fatigue syndrome.[1-5] Although cyclooxygenase inhibitors can often suppress hyperalgesia, their well known dangerous side effects – GI bleeds and renal damage with non-specific cox inhibitors, increased risk for vascular events with cox-2-specific agents – render them less than ideal for control of chronic hyperalgesia.[6] And, whereas acute administration of opiates blocks pain transmission, opiate tolerance tends to develop during long-term use, and can even evolve to hyperalgesia.[7-9] Hence, safer strategies for coping with chronic hyperalgesia are needed.

In this regard, it is pertinent to observe that three phenomena participate in the mediation of hyperalgesia, often at multiple levels: induction of oxidative stress, stemming from NADPH oxidase and/or mitochondria; activation of NF-kappaB; and induction of cox-2, leading to increased production of PGE2

and activation of EP1 and EP4 receptors. Each of these phenomena is potentially addressable with nutraceuticals or drugs that are safer and less problematic than cyclooxygenase inhibitors and opiates – namely, phycocyanobilin, salsalate, and beta-boswellic acid, respectively.

A Mediating Role for Oxidative Stress in Diverse Hyperalgesia Syndromes

Oxidative stress in inflamed peripheral tissues helps to drive the production of pro-inflammatory cytokines and prostanoids that triggers inflammatory hyperalgesia. In primary sensory neurons, oxidative stress originating in the dorsal root ganglion (DRG) from Nox1 or Nox4 has been shown to play a mediating role in both inflammatory and neurogenic hyperalgesia.[10-12] Oxidative stress in primary sensory neurons may promote hyperalgesia by boosting expression of the nocireceptor TRPV1, and by up-regulating activation of PKC isoforms (notably PKCe) that phosphorylate TRPV1, sensitizing it to activation.[10, 13] Oxidative stress in the substantia gelatinosa of the spinal cord dorsal horn – where the cell bodies of the secondary spinal afferents are located - has been observed in inflammatory, neurogenic, and morphine-induced hyperalgesia, and intrathecal administration of a wide range of antioxidant compounds – NADPH oxidase inhibitors, superoxide dismutase mimetics, and peroxynitrite decomposition catalysts – can suppress hyperalgesia.[8, 14-19] This dorsal horn oxidative stress appears to arise primarily from Nox2 in activated microglia, and promotes hypersensitization of spinal sensory neurons by inducing glial production of cytokines TNF-alpha and IL-1beta, which act directly or indirectly on these neurons.[14, 20, 21] In capsaicin-induced and neurogenic hyperalgesia, increased superoxide production in the mitochondria of dorsal horn neurons has been observed, likely stemming from an increase in intracellular calcium reflecting NMDA receptor overactivation.[22, 23] Dorsal horn oxidative stress promotes hyperalgesia, at least in part, by inhibiting GABA release within the substantia gelatinosa; GABAergic neurons in this region function to hyperpolarize the dendrites of secondary spinal afferents, reducing their sensitivity to activation.[15] Cytokines also act on spinal sensory neurons to promote phosphorylation of the NR1 subunit of NMDA receptors, which up-regulates their activity.[18]

The oxidative species which mediate hyperalgesia appear to be diverse. The hydrogen peroxide stemming from superoxide dismutation up-regulates the activation of NF-kappaB and the induction of cox-2, iNOS, and pro-inflammatory cytokines, and may work in additional ways to modulate pain reception by altering the redox status of signaling proteins. But peroxynitrite and possibly superoxide per se also clearly play distinctive roles in hyperalgesia induction, as demonstrated by the efficacy of superoxide dismutase mimetics and peroxynitrite decomposition catalysts, whether administered systemically or intrathecally, for controlling hyperalgesia.[16, 24] Salvemini and colleagues have conducted a number of pertinent studies in this regard, and have catalogued a range of mechanisms whereby peroxynitrite might work to boost the glutamate signaling that triggers activation of the secondary spinal afferents.[24] Peroxynitrite, arising in the dorsal horn, appears to be a key mediator of the hyperalgesia induced by the neurotoxic anti-cancer drug paclitaxel.[25]

Phycocyanobilin and Complementary Antioxidants May Aid Control of Hyperalgesia

Oxidative and nitroxidative stress are clearly dependent on a source of superoxide, and usually reflect superoxide overproduction. Oxidative stress stemming from NADPH activation – Nox1 and Nox4 in primary afferent neurons, and Nox2 in microglia – is clearly a key driver of hyperalgesia.[10-12, 14, 17] It is therefore of great interest that free intracellular bilirubin (generated by heme oxygenase activity) functions physiologically to inhibit certain NADPH oxidase complexes; the isoform-specificity of this

effect requires further clarification.[26-29] Moreover, it appears that the biliverdin derivative phycocyanobilin (PhyCB) – richly supplied by cyanobacteria such as spirulina, where it occurs as a covalently-bound chromophore of the light-harvesting protein phycocyanin – can mimic NADPH oxidase inhibitory activity of bilirubin, even when administered orally; this likely accounts for the potent and versatile anti-inflammatory activity of oral or parenteral phycocyanin demonstrated in a wide range of rodent inflammation models.[30-32] It appears likely that PhyCB has access to the central nervous system and spinal cord, inasmuch as phycocyanin or whole spirulina shows utility in rodent models of Parkinson's disease, epilepsy, premature dementia, and stroke.[33-37] Hence, it is reasonable to suspect that oral administration of PhyCB/phycocyanin may have an ameliorative impact on hyperalgesia, both by helping to quell the inflammation that triggers inflammatory hyperalgesia, and by impacting the oxidative stress that acts directly or indirectly to hypersensitize primary and secondary sensory afferents.[38]

Although, as noted, phycocyanin has exerted anti-inflammatory impacts in a large number of rodent studies, only one study to date has reported its impact on hyperalgesia per se. This study showed that parenterally-administered phycocyanin suppresses the hyperalgesia induced by the injection of carrageenan into the paws of rats.[39] Since phycocyanin administration substantially quelled the paw inflammation – as indicated by reduced induction of iNOS and cox-2 and production of pro-inflammatory cytokines in the affected paws – this study unfortunately does not allow us to judge whether PhyCB can also influence hyperalgesia via antioxidant effects in the DRG and dorsal horn. It therefore would be of great interest to examine the impact of phycocyanin on alternate forms of hyperalgesia – notably that induced by peripheral nerve damage, opiate administration, capsaicin injection, or neurotoxic chemotherapies. Despite the existence of an ample rodent literature, clinical research with spirulina or phycocyanin is still in an embryonic stage, and it is not yet possible to state with assurance what intakes of spirulina or phycocyanin might be required for systemic control of NADPH oxidase activity; extrapolation from rodent studies suggests that an intake of 15-30 g spirulina daily might be needed for an optimal antioxidant effect, on the presumption that humans metabolize PhyCB in a manner roughly comparable to rodents.[30]

Glutathione can function in multiple ways to counteract the impact of oxidative stress. Rossato and colleagues have demonstrated that levels of glutathione in the lumbar spine are reduced in rats exhibiting hyperalgesia triggered by hind paw injection of capsaicin; they further showed that systemic or intrathecal administration of N-acetylcysteine (NAC) – which boosts intracellular glutathione synthesis by providing the rate-limiting substrate cysteine – alleviated hyperalgesia in these rats, whereas administration of a drug that inhibits glutathione synthesis (buthionine suphoxamine) potentiated the hyperalgesia.[40] Oral administration of NAC has also been shown to alleviate neurogenic thermal hyperalgesia in rats.[41] In light of evidence that intracellular glutathione levels and the efficiency of glutathione synthesis tend to decline with advanced age, optimizing cysteine availability through NAC administration (several grams daily) might prove to be advantageous in elderly subjects afflicted with hyperalgesia.[42] Further evaluation of NAC in rodent models of hyperalgesia clearly would be appropriate.

Phase 2 inducers and melatonin have the potential to boost the expression of antioxidant enzymes – while also enhancing glutatathione synthesis – in neural tissue. They therefore may have potential for control of hyperalgesia, as suggested by a few rodent studies.[14, 43-47] Complementing phycocyanin administration with adjuvant antioxidant measures such as NAC, melatonin, and/or phase 2 inducers (such as lipoic acid)

might be appropriate – particularly if superoxide of mitochondrial origin contributes meaningfully to the pathogenesis of hyperalgesia.

Salicylate Might Impact Hyperalgesia via Inhibition of NF-kappaB Activity

A key way in which oxidative stress provokes inflammation and hyperalgesia is via up-regulation of the activity of pro-inflammatory transcription factors such as AP-1 and NF-kappaB.[48, 49] NF-kappaB activation plays a key role in induction of cox-2, iNOS, and a range of cytokines that mediate inflammation and can also induce hypersensitization of spinal afferent neurons.[50] Activation of NF-kappaB in the spinal column has been observed in both inflammatory and neurogenic hyperalgesia, and the utility of intrathecally administered NF-kappaB antagonists for controlling this hyperalgesia demonstrates that spinal NF-kappaB activation – likely in microglia, as abetted by Nox2 activity – plays a mediating role in these syndromes.[51-62] Obviously, NF-kappaB antagonists could also influence inflammatory hyperalgesia by favorably impacting peripheral inflammation.

High doses of salicylate – most appropriately administered clinically as the better-tolerated dimer salsalate – have long been used effectively and safely in the treatment of inflammatory syndromes, most notably rheumatoid and osteo-arthritis.[63-66] Since salicylate, unlike its derivative aspirin or the NSAID drugs, produces only a weak and transitory inhibition of cyclooxygenases that is of little physiological or clinical significance, its utility in inflammation evidently stems from another mechanism.[67] The discovery that clinically achievable concentrations of salicylate can suppress the canonical pathway of NF-kappaB activation via direct inhibitory binding to IkappaB kinase-beta (IKKb), casts a new light on salicylate's clinical utility.[68, 69] Arguably, a portion of the pain relief achieved by salicylate therapy in arthritis and other inflammatory disorders might reflect suppression of hyperalgesia via inhibition of NF-kappaB activity in microglia of the dorsal horn (and possibly primary afferents in the DRG). Unfortunately, there do not appear to be any studies that have tested this possibility in rodent models.

The safety of salicylate therapy reflects the fact that it does not meaningfully inhibit cyclooxygenase activity.[67] The dose-limiting side effect of salicylate is fully reversible ototoxicity, manifesting as tinnitus and mild hearing loss.[70-72] Most patients tolerate the standard dose of salsalate – 3 g daily, 1.5 g b.i.d. – without notable ototoxicity, but this side effect becomes more prominent if the dose is pushed to 4.5 g daily. Mild gastrointestinal upset – not associated with GI bleeding – is occasionally reported with salicylate therapy, but this side effect is minimized when salicylate is administered as salsalate.[73, 74] (The latter is insoluble in the acidic stomach, and hence does not enter the gastric mucosa; in the alkaline intestine, it solubilizes and is cleaved by esterases to generate free salicylate, which is then absorbed.)

At 3 grams daily, salsalate will achieve at best a very partial inhibition of NF-kappaB activation. This is not necessarily a bad thing, as an intense inhibition of NF-kappaB would be expected to negatively impact immune defenses. But it does suggest that a more effective control of inflammation and hyperalgesia might be expected if salsalate therapy were complemented with appropriate adjuvant measures such as PhyCB and (as discussed below) boswellic acids.

Beta-Boswellic Acid as a Safe Alternative to Cox Inhibitors

The well documented utility of NSAIDs in hyperalgesias reflects the fact that oxidative stress, NF-kappaB activation and cytokine exposure can up-regulate cox-2 expression in peripheral tissue, primary

sensory afferents, and microglia of the dorsal horn. The PGE2 which is a downstream product of cox-2 activity promotes hypersensitivity of primary afferent neurons via activation of EP1 and EP4 receptors, which in turn increase the activity of PKC and adenyl cyclase, respectively.[75] Both PKC and PKA induce serine phosphorylations of TRPV1 that up-regulate its activity; and these kinases can act on additional ion channels and receptors in primary afferents in ways that promote hyperalgesia. Intrathecal administration of PGE2 induces thermal and mechanical hyperalgesia in rats, and this effect is blocked by co-administration of selective EP1 inhibitors; hence, PGE2 produced by activated spinal microglia likely contributes to hyperalgesia.

Three different enzymes are capable of converting the PGH2 generated by cyclooxygenases to PGE2. Microsomal prostaglandin E synthase-1 (mPGES-1) tends to be co-induced with cox-2, and appears to be functionally coupled to cox-2 in generation of PGE2.[76, 77] mPGES-1 knockout mice are substantially protected from inflammatory hyperalgesia (as evoked by i.p. injection of acetic acid, or paw injection of collagen).[78] There is credible speculation that drugs which selectively inhibit mPGES-1 could provide a much safer alternative to NSAIDs in the management of inflammation, inasmuch as such drugs would not impede prostacyclin production, or boost leukotriene synthesis.[77, 79] Propitiously, mPGES-1 knockout mice do not experience the GI or renal damage provoked by NSAIDs.[79] When these mice are crossbred with LDL receptor knockout mice, the double knockouts that result are less prone to atheroma, and the atheroma that is observed tends to be more stable, than in LDL receptor knockouts with wild-type mPGES-1 activity; this suggests that mPGES-1 inhibition would not be attended by the increased cardiovascular risk observed during cox-2 inhibitor therapy.[80]

The gum resin of boswellia serrata trees – a.k.a. frankincense – is a rich source of pentacyclic triterpenes known as boswellic acids. Frankincense has traditionally been used in Ayurvedic medicine as an anti-inflammatory therapy, and extracts rich in boswellic acids do indeed demonstrate anti-inflammatory activity in rodents.[81-83] Pilot clinical trials also suggest the utility of such extracts in rheumatoid and osteoarthiritis, colitis, and radiation-provoked brain edema.[84-89] Although initial studies suggested that keto-boswellic acids might be acting clinically as inhibitors of 5-lipoxygenase, more recent analyses discount this view.[90, 91] Rather, there is recent evidence that clinically achievable concentrations of beta-boswellic acid (which constitutes about one-third of the total boswellic acids in frankincense) can inhibit mPGES-1.[92] Quite likely, this explains the anti-inflammatory impact of boswelllic acid preparations clinically and in rodents.

To date, there have been no studies examining the impact of boswellic acids on hyperalgesia in rodents; in light of the recent evidence that beta-boswellic acids can target mPGES-1, such studies appear to be warranted. The toxicology of boswellic acids in rodents is benign, and no serious side effects have been reported in pilot clinical studies with these agents; aside from mild GI upset reported by a minority of patients when high doses are ingested, boswellic acids appear to be well tolerated.[91, 93, 94] Moreover, beta-boswellic acid has been found to have good access to the CNS when administered orally to rodents.[95] Therefore, if they can indeed function clinically as inhihitors of mPGES-1, they may have good potential for control of hyperalgesia. Most commercial nutraceutical sources of boswellic acids provide about 200 mg of total boswellic acids per capsule; an examination of the pilot clinical literature with boswellic acids suggests that 2-3 of these capsules, twice daily, might be required for useful clinical activity.

Summing Up

In overview, the foregoing discussion suggests that a regimen comprised of adequate doses of PhyCB (as provided by phycocyanin or whole spirulina), salsalate, and beta-boswellic acid (from boswellia gum resin extracts) may have the potential to achieve safe control of various types of hyperalgesia via complementary inhibition of NADPH oxidase, NF-kappaB, and mPGES-1, respectively. NAC and melatonin could be included to complement the antioxidant activity of PhyCB. Salsalate could be omitted from this regimen for patients who are highly sensitivity to salicylate-induced ototoxicity. Each of these agents requires further evaluation in rodent models of hyperalgesia, and their combined activity could also be assessed in such models. It would also be of interest to test such a regimen in models of morphine intolerance and morphine-induced hyperalgesia.

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