Exploiting Injury-Induced Peripheral Opioid Receptor Changes in Novel Analgesic Development for Chronic Pain

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INTRODUCTION

The process of novel analgesic drug development, from ideation to sustainable marketing, has many steps: each with multiple challenges. Successful evolution to safe, effective, and economically viable clinical use requires a series of positive outcomes in both scientific and business arenas. Failure at any point can result in the abandonment of further development of the new molecular entity (NME). In fact, most early analgesic development approaches end well before regulatory approval and marketing for clinical use. Only 2% of NMEs for pain transitioned to Food and Drug Administration (FDA) approval compared with 10% for all diseases from 2007 through 2017 (1).

When one considers the enormity of the problems associated with the most potent currently available analgesics, opioids, the urgency of the unmet medical need cannot be overstated (2). Still non-opioid options, especially in the US, are shrinking rather than growing. The removal of marketed cyclooxygenase-2 (COX-2) inhibitors from the US market (3) and other jurisdictions, and the failure of approval for newer COX-2 agents (4) have made this situation even more acute. With few NMEs coming online, development has focused on recycling already approved traditional opioids with novel delivery systems (5, 6) or abuse deterrent formulations (7). A biased mu opioid agonist, oliceridine, is among the few NMEs to reach regulatory approval in recent years (8). While it does provide an improved safety profile with respect to respiratory depression (9), like the other mu opioids, this remains a risk. Moreover, mu opioids, as centrally acting agents, carry with them the additional risk of addiction (10). According to the Centers for Disease Control and Prevention (CDC), 1 out of 4 persons receiving prescription opioids suffer from addiction, most having experienced their first opioid in the form of a prescription from a physician (11).

Expanding the physicians' armamentarium to address moderate-to-severe chronic pain beyond archetypal mu opioids is an urgent unmet need. Chronic pain, by some estimates, affects nearly one-third of the population worldwide (12). Yet it is important to note that failure to address this therapeutic gap has not been the result of apathy or lack of effort on the part of pharma or the scientific community. Numerous NMEs have shown great promise in animal models only to fail in clinical trials. Some failures may have conceivably been attributed to failure to reach effective concentrations sufficient for target engagement due to inappropriately low dosing or suboptimal formulation. Historically, there were undoubtedly other failures that might well have been avoided by applying the same rigor to preclinical investigations that has been recognized as good practice in clinical trials for many years (13). Despite considerable advances directed toward both the conduct and reporting of preclinical trials (14–16), difficulties in translation to the human pain state remain.

The thesis presented here is that there are at least two more major knowledge gaps that have hampered successful development of analgesic NMEs. Urgently needed are: (1) better preclinical pain models and pain measurement methods (or methods to eliminate animal models altogether), and (2) a better understanding of the alterations in pain pathways associated with chronic pain states. These two areas requiring improved understanding are interrelated. While often assumed that specific behavioral observations and responses to various stimuli reflect pain in preclinical models, this is often far from certain. However, since preclinical models aim to emulate specific chronic pain conditions, ensuring similar underlying mechanisms are present in both the preclinical and the clinical models strengthens confidence in the ability to monitor analgesic response in animals unable to provide self-report.

PRECLINICAL PAIN MODEL TRANSLATION

The current regulatory process involves preclinical small animal efficacy testing (usually rodents), followed by both small and larger animal (typically canine) safety testing, before Phase I clinical trials on healthy volunteers. Efficacy and safety investigations in humans who suffer from the specific pain indication(s) of interest begin much later in Phase II and III clinical trials after many millions of dollars have already been spent (17). Failure at this late stage results in a dampening effect on investment and on the enthusiasm for the entire process. The inclusion of Proof-of-Concept (PoC) and Proof-of-Activity (PoA) studies early in clinical development might help identify biomarkers for better candidate selection in subsequent Phase II and II trials, thus improving outcomes (18). The use of biomarkers, especially in clinical phase I and II have been estimated to potentially double the chance of successful FDA approval (19).

Biomarkers can take the form of genetic, physical or laboratory testing, neuroimaging, or other observations that can be correlated with diagnostic, prognostic, or therapeutic responses (20). Often mentioned in reference to clinical chronic pain conditions is the use of quantitative sensory testing (QST). QST is particularly useful in chronic pain states affecting sensory perception thresholds and tolerability (e.g., hyperalgesia, allodynia, hyperpathia). Neuroimaging, while challenging logistically, could also be of value in both preclinical and clinical models. Genetic testing is appealing, but interspecies differences limit generalizability. Skin biopsy is feasible in both preclinical and clinical models. In conditions associated with peripheral small-fiber neuronal changes, intra-epidermal nerve fiber density examination has been used for diagnostic, pharmacodynamic response, predictive, and prognostic purposes (21, 22). Situations where injury-induced changes in opioid receptor density or behavior are expressed in affected tissues in the periphery lend themselves to this latter PoC approach. Biopsy to directly assay peripheral opioid receptor density has been performed in the preclinical setting (23). For clinical study, less invasive approaches may be desirable. Indirect or surrogate studies assessing the response to local challenge with chemically

(e.g., capsaicin), thermally (e.g., UV: ultraviolet radiation) or electrically evoked stimuli can be useful in first-in-human drug studies to document activity (PoA) (24), and potentially in patient selection for later clinical studies to differentiate candidates with preexisting inflammation-induced hyperalgesic skin from less susceptible candidates in select clinical settings. Accordingly, a more effective preclinical process, incorporating relevant biomarkers, should also result in greater success. However, unless and until methods are developed that will be accepted by regulatory authorities that obviate the need for animal studies, proactively attempting to improve the translation from lower species to humans remains critical.

This first issue, interspecies differences, has been concisely described elsewhere (25). Briefly, humans are not large rats, and unfortunately even rats are not just large mice. Expecting rodents to express behaviors analogous to complex pain characteristics associated with chronic pain in humans, such as the emotional/affective and suffering components, is not reasonable given the anatomic differences in forebrain structure. These authors also make the important point that chronic pain expression, as assessed by behavioral changes in rodents, is further complicated by the fact that rodents are prey animals. Visible evidence of vulnerability in a prey animal is not conducive to survival and thus the reluctance to show signs of weakness following injury is more adaptive than stoic. Altered burrowing behavior may be more indicative of distress than other observations such as weight-bearing, gait abnormalities or other spontaneous behaviors presumed to relate to pain (26, 27). The use of small non-human primates (28, 29), if accepted by regulatory bodies, could potentially improve translation, especially if naturally occurring pain conditions provided an ethical opportunity for study. While much can be gleaned from human *ex-vivo* human tissue regarding mechanism (30, 31), response to a novel treatment can only be ascertained *in vivo*. Moreover, the clinical human population, far from a specific strain of animal selected to create uniformity in preclinical testing, are more like "wild type" animals. The diverse responses to analgesics reflect this fact, again emphasizing the importance of biomarkers in subject selection in clinical trials, as well as the use of biomarkers in the earlier preclinical studies (22).

It is well known that the "standard" preclinical pain models used to emulate various human chronic pain conditions produce idiosyncratic responses (such as altered licking, vocalization, facial expressions, weight-bearing, feeding, social interaction, ambulation, rearing, burrowing, and conditioned place preference) that are assumed to relate to the pain experience (32). These models imperfectly match clinical conditions with respect to the inciting injuries, longitudinal time course following injury, and genetic predisposition (33). Similarly, typical interventions imperfectly match duration of treatment, as most preclinical efficacy trials involve a single or a limited number of repeat doses. Nor can they, as limited duration studies, account for subsequent time-dependent adaptation, including the development of tolerance to intervention. Longer-term studies are performed in safety testing; applying this same strategy to efficacy testing could potentially improve success in the clinical setting.

By way of example, the types of injuries that are commonly used to study potential treatments for neuropathic pain include placing ligatures around the sciatic nerve (chronic constriction injury; CCI), ligating a portion of the sciatic nerve (partial nerve injury; PNI), ligation of spinal nerves contributing to the sciatic nerve (spinal nerve ligation; SNL), and ligation of two of the three terminal branches of the sciatic nerve (spared nerve injury; SNI). These models develop on a different time courses, last different periods of time, result in differing degrees of evoked behaviors such as mechanical hyperalgesia, mechanical allodynia, and thermal hyperalgesia, and exhibit differences in spontaneous behaviors as well (34). A model focusing on the initiation of neuroinflammation without overt nerve injury has been proposed whereby the sciatic nerve is surrounded by a cuff that bathes the nerve in zymosan (Sciatic inflammatory neuritis; SIN). However, it still involves surgical incision (itself a confounding injury) and implantation of the foreign body (35). A variation on this approach whereby the zymosan is injected percutaneously with no surgical incision or implanted foreign body other than the zymosan has been described (36). These latter two approaches have not been widely accepted, but arguably are less contrived. Additionally, contralateral effects in the non-surgical percutaneous model, as in the clinical condition, were largely absent, in contrast to the dose-dependent contralateral effects in SIN and the less prominent but still consistently reproducible contralateral effects noted in CCI (37). Regardless, clinical neuropathic pain conditions only rarely involve injuries due to suture or section of all or part of the sciatic nerve, its roots, or its branches. The models do, however, allow for examination of features that may relate to symptoms experienced in some patients. Back translating from human to animal by identifying patient phenotypes based on QST or other biomarkers has been suggested as a "precision medicine" method to improve preclinical pain model selection (38). Absent better models, this approach seems completely reasonable.

PERIPHERAL OPIOID RECEPTOR CHANGES

Just as humans are not large rodents, somewhat analogously chronic pain is not persistent acute pain. Chronic pain often involves altered pain pathways. Long-term potentiation, microglial activation, synaptic pruning and other mechanisms contribute to the neuroplasticity that can cause pain to become intransient and independent of the initial inciting injury. In these cases, chronic pain becomes a disease in and of itself (39, 40).

Given the importance of mu opioid receptors (MOR) within principal pain pathways in the central nervous system (brain and spinal cord), up- or down-regulation of MORs in number or function impacts the ability of traditional mu opioids to modulate chronic pain states. Furthermore, in some cases, alterations in MOR activity in chronic pain states appear to relate to specific insults. Several common chronic pain states are recognized to be relatively unresponsive to mu opioid agonists

and characteristically difficult to treat, including bone cancer, fibromyalgia, and neuropathic pain.

MORs are down-regulated in bone cancer (41). Alternatively, multiple non-opioid pain pathways are activated, including enhanced involvement of inflammatory mediators such as bradykinin (42). In fibromyalgia, investigators have described reduced central availability of MORs (43). Neuropathic pain states are similarly characterized by a shift away from mu-opioid dominated pathways to noradrenergic pathways (44). The lack of efficacy in these situations contributes to inappropriate and futile dose escalation. Consequently, these phenotypic shifts, along with alterations due to the act of mu agonist administration inducing tolerance and addiction, are among the reasons that traditional mu opioids have not been shown to be consistently superior to non-opioid analgesics in chronic pain states (45, 46).

In contrast, injuries resulting in peripheral inflammation, at later stages, eventually induce migration of MORs into peripheral tissues (47, 48), offering the opportunity for mu opioid agonists to have distinct analgesic effect apart from their action in the central nervous system. Analgesics, including mu opioids, that remain in the periphery avoid central MOR-mediated sedative effects, respiratory depression, and the potential for addiction. Unfortunately, mu opioid agonists can themselves induce microglial activation that induces hyperalgesia, lowers pain thresholds, and induces a primed microglial phenotype that persists even after opioid discontinuation, thus worsening rather than alleviating chronic pain (49).

Inflammation also induces changes in non-mu opioid receptor activity in the periphery. Kappa opioid receptors (KOR) are constitutively present peripherally and may participate in anti-inflammatory induced analgesia (50). However, even absent inflammation, peripheral KORs actively mediate analgesia (51). Yet in the presence of inflammation, kappa opioid-mediated analgesia is enhanced. This enhancement likely results, at least in part, from synergistic action on delta opioid receptors (DOR). The DOR, also constitutively present in the periphery, is normally quiescent, being functionally impeded by G Protein-Coupled Receptor Kinase-2 (GRK2). In the presence of inflammation, the DOR receptor becomes active. Proinflammatory bradykinin stimulates GRK2 movement away from DOR and onto Raf Kinase Inhibitory Protein (RKIP). Protein kinase C (PKC)-dependent RKIP phosphorylation associated with the binding of bradykinin (BK) induces this GRK2 sequestration, restoring DOR functionality in sensory neurons (52). Additionally, KORs and DORs form heterodimers. The activity of these heterodimers has been demonstrated in peripheral sensory neurons and the allosteric interaction between the kappa and delta components of the heterodimers is thought to contribute to the enhancement of kappa-mediated analgesia by delta agonists (53).

DISCUSSION

Adaptation to both injury and exposure to exogenous toxins can be essential for survival. Drugs, as exogenous substances, can be considered potentially toxic assaults to be pharmacokinetically

metabolized and eliminated. Perhaps less well appreciated are altered pathways that pharmacodynamically minimize the drug's effects though the induction of tachyphylaxis, tolerance, or other mechanisms. These processes can become even more significant with repeated administration, as is required in chronic conditions. Thus, over time these adaptations may severely impair the efficacy of the administered drug. Agents that appear to be effective in short-term preclinical models may subsequently fail with longer-term administration clinically. In contrast, when the altered pathways responsible for these effects are understood, they can be exploited to improve drug efficacy in specific conditions. The phenotypic shift in neuropathic pain, away from opioid-mediated pathways in favor of noradrenergic pathways, as previously mentioned, provides just such an opportunity. Further, because adrenergic receptors are up-regulated in peripheral sensory neurons (54), they are readily accessible for study. This well-known and long studied observation could have been exploited as a useful biomarker for screening and recruiting susceptible candidates for inclusion in clinical trials, as well as optimizing the selection of preclinical models for detailed study.

Another NME among the very few novel analgesics to be FDA approved in recent decades is tapentadol. It is one of the multiple potential analgesics demonstrating significant noradrenergic effects, suggesting utility in neuropathic pain states (55). Mechanistically, tapentadol's relatively modest analgesic activity attributed to action at the MOR is greatly magnified by synergistic noradrenergic reuptake inhibition (56). While the effect on descending central pathways may be largely responsible for its non-opioid analgesic effect, had initial preclinical and early clinical trials focused on noradrenergic receptor upregulation, as a peripherally accessible biomarker, the development process might well have been accelerated Ultimately, tapentadol extended release was approved for neuropathic pain associated with diabetic peripheral neuropathy (57, 58).

Many chronic pain conditions, including neuropathic pain, involve the initiation of inflammatory pathways, albeit to varying degrees. One NME, CAV1001, has demonstrated efficacy in multiple preclinical models (59). CAV1001, as a dual-acting, peripherally restricted kappa/delta opioid agonist, was more effective in reducing inflammatory-induced hyperalgesia in mice by an order of magnitude when compared on an equimolar basis to a peripherally restricted pure kappa opioid agonist. This synergy is consistent with DOR becoming active in the inflammatory state and consequent potential involvement

of DOR/KOR heterodimers. Moreover, it was shown to be relatively more potent in preclinical models known to have greater involvement of the inflammatory cascade (59). The use of a biomarker designed to identify heterodimers of DOR/KOR and their behavior following injury in the selection of candidates for subsequent clinical trials could greatly improve the odds for successful clinical development of this NME. Absent immunostaining or other practical technique to directly assess peripheral receptor density in sensory neurons from skin biopsy, indirect evidence for inflammation-induced hyperalgesic responses could be used.

UV-evoked inflammatory hyperalgesia has been used as an early biomarker for efficacy in early human studies on volunteers. Although the technique causes no significant injury, it does result in the local elaboration of multiple inflammatory mediators (24, 60). This model has been used experimentally in human volunteers to confirm efficacy for multiple drugs, including opioids (61). Importantly, the technique has also been successfully used in preclinical translational models (62). Additional application of laser algesimetry, where a CO_2 laser stimulus is used to generate somatosensory evoked potentials, can add an objective measure of efficacy that has been used in healthy volunteers with UV-induced inflammatory hyperalgesic skin (24) and might be useful in clinical trial subject screening. Numerous drugs have been evaluated with this technique including mu-, kappa- and the mixed-opioid agonists tramadol, tapentadol, and pentazocine.

While the aforementioned factors are important, they illustrate just two among many explanations as to why most seemingly promising preclinical drugs go on to fail in clinical trials. The examples given also reinforce the concept that no single analgesic can be expected to be useful for all chronic pain states, and that mechanism based multimodal approaches will remain best practice for the foreseeable future. Novel analgesic development should focus on a customized, personalized, biomarker driven approach that fully considers not only very specific mechanisms of action, but also the underlying pathophysiology as it exists within each genetically and epigenetically unique chronic pain patient.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

REFERENCES

1. Thomas D, Wessel C. *The State of Innovation in Highly Prevalent Diseases: Vol II: Pain and Addition Therapeutics. BIO Industry Analysis.* (2018). Available at: http://go.bio.org/rs/490-EHZ-999/images/BIO_HPCP_Series-Pain_Addiction_2018-02-08.pdf (accessed Febru2022).

2. Degenhardt L, Grebely J, Stone J, Hickman M, Vickerman P, Marshall BDL, et al. Global patterns of opioid use and dependence: population harms, interventions, and future action. *Lancet.* (2019) 394:1560–79. doi: 10.1016/S0140-6736(19)32229-9

3. Cahana A, Mauron A. The story of Vioxx—no pain and a lot of gain: ethical concerns regarding conduct of the pharmaceutical industry. *J Anesth.* (2006) 20:348–51. doi: 10.1007/s00540-006-0432-7

4. Viscusi ER. Frenkl TL, Hartrick CT, Rawal N, Kehlet H, Papanicolaou D, et al. Perioperative use of etoricoxib reduces pain and opioid side-effects after total abdominal hysterectomy: a double-blind, randomized, placebo-controlled phase III study. *Curr Med Res Opin.* (2012) 28:1323–35. doi: 10.1185/03007995.2012.707121

5. Hartrick CT, Bourne MH, Gargiulo K, Damaraju CV, Vallow S, Hewitt DJ. Fentanyl iontophoretic transdermal system for acute-pain

management after orthopedic surgery: a comparative study with morphine intravenous patient-controlled analgesia. *Reg Anesth Pain Med.* (2006) 31:546–54. doi: 10.1016/j.rapm.2006.08.011

6. Hartrick CT, Martin G, Kantor G, Koncelik J, Manvelian G. Evaluation of a single-dose, extended-release epidural morphine formulation for pain after knee arthroplasty. *J Bone Joint Surg Am.* (2006) 88:273–81. doi: 10.2106/JBJS.D.02738

7. Litman RS, Pagán OH, Cicero TJ. Abuse-deterrent opioid formulations. *Anesthesiology.* (2018) 128:1015–26. doi: 10.1097/ALN.0000000000002031

8. Markham A. Oliceridine: First Approval. *Drugs.* (2020) 80:1739–44. doi: 10.1007/s40265-020-01414-9

9. Soergel DG, Subach RA, Burnham N, Lark MW, James IE, Sadler BM, et al. Biased agonism of the μ-opioid receptor by TRV130 increases analgesia and reduces on-target adverse effects versus morphine: a randomized, double-blind, placebo-controlled, crossover study in healthy volunteers. *Pain.* (2014) 155:1829–35. doi: 10.1016/j.pain.2014.06.011

10. Hartrick CT, Gatchel RJ, Conroy S. Identification and management of pain medication abuse and misuse: current state and future directions. *Expert Rev Neurother.* (2012) 12:601–10. doi: 10.1586/ern.12.34

11. *Centers for Disease Control and Prevention.* (2019). Available at: https://www.cdc.gov/opioids/basics/prescribed.html (accessed February 2022).

12. Cohen SP, Vase L, Hooten WM. Chronic pain: an update on burden, best practices, and new advances. *Lancet.* (2021) 397:2082–97. doi: 10.1016/S0140-6736(21)00393-7

13. Schulz KF, Altman DG, Moher D, CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Ann Intern Med.* (2010) 152:726–32. doi: 10.7326/0003-4819-152-11-201006010-00232

14. Clark JD. Preclinical pain research: can we do better? *Anesthesiology.* (2016) 125:846–9. doi: 10.1097/ALN.0000000000001340

15. Andrews NA, Latrémolière A, Basbaum AI, Mogil JS, Porreca F, Rice ASC, et al. Ensuring transparency and minimization of methodologic bias in preclinical pain research: PPRECISE considerations. *Pain.* (2016) 157:901–9. doi: 10.1097/j.pain.0000000000000458

16. du Sert NP, Hurst V, Ahluwalia A, Avery MT, Baker M, Browne WJ, et al. The ARRIVE guidelines 20: Updated guidelines for reporting animal research. *PLoS Biol.* (2020) 18:e3000410. doi: 10.1371/journal.pbio.3000410

17. Sertkaya A, Wong H-H, Jessup A, Beleche T. Key cost drivers of pharmaceutical clinical trials in the United States. *Clin Trials.* (2016) 13:117–26. doi: 10.1177/1740774515625964

18. Hijma HJ, Groeneveld GJ. Analgesic drug development: proof-of-mechanism and proof-of-concept. *Med Drug Discov.* (2021) 10:100083. doi: 10.1016/j.medidd.2021.100083

19. Wong CH, Siah KW, Lo AW. Estimation of clinical trial success rates and related parameters. *Biostatistics.* (2019) 20:273–86. doi: 10.1093/biostatistics/kxx069

20. Marchi A, Vellucci R, Marmeli S, Piredda AR, Finco G. Pain biomarkers. *Clin Drug Investig.* (2009) 1:41–6. doi: 10.2165/0044011-200929001-00006

21. Smith SM, Dworkin RH, Turk DC, Baron R, Polydefkis M, Tracey I, et al. The potential role of sensory testing, skin biopsy, and functional brain imaging as biomarkers in chronic pain clinical trials: IMMPACT considerations. *J Pain.* (2017) 18:757–77. doi: 10.1016/j.jpain.2017.02.429

22. Tracey I, Woolf CJ, Andrews NA. Composite pain biomarker signatures for objective assessment and effective treatment. *Neuron.* (2019) 101:783–800. doi: 10.1016/j.neuron.2019.02.019

23. Zambelli VO. Fernandes ACdO, Gutierrez VP, Ferreira JCB, Parada CA, et al. Peripheral Sensitization Increases Opioid Receptor Expression and Activation by Crotalphine in Rats. *PLoS ONE.* (2014) 9:e90576. doi: 10.1371/journal.pone.0090576

24. Chizh BA, Priestly T, Rowbotham M, Schaffler K. Predicting therapeutic efficacy—experimental pain in human subjects. *Brain Res Rev.* (2009) 60:243–54. doi: 10.1016/j.brainresrev.2008.12.016

25. Yezierski RP, Hansson P. Inflammatory and neuropathic pain from bench to bedside: what went wrong? *J Pain.* (2018) 19:571–88. doi: 10.1016/j.jpain.2017.12.261

26. Vierck CJ, Yezierski RP. Comparison of operant escape and reflex tests of nociceptive sensitivity. *Neurosci Biobehav Rev.* (2015) 51: 223–42. doi: 10.1016/j.neubiorev.2015.01.022

27. Deuis JR, Dvorakova LS, Vetter I. Methods used to evaluate pain behaviors in rodents. *Front Mol Neurosci.* (2017) 10:284. doi: 10.3389/fnmol.2017.00284

28. Hama AT, Toide K, Takamatsu H. Beyond rodent models of pain: non-human primate models for evaluating novel analgesic therapeutics and elaborating pain mechanisms. *CNS Neurol Disord Drug Targets.* (2013) 12:1257–70. doi: 10.2174/18715273113129990111

29. Ezran C, Karanewsky CJ, Pendleton JL, Sholtz A, Krasnow MR, Willick J, et al. The mouse lemur, a genetic model organism for primate biology, behavior, and health. *Genetics.* (2017) 206:651–64. doi: 10.1534/genetics.116.199448

30. McGuire C, Boundouki G, Hockley JRF, Reed D, Cibert-Goton V, Peiris M, et al. Ex vivo study of human visceral nociceptors. *Gut.* (2018) 67:86–96. doi: 10.1136/gutjnl-2016-311629

31. Moy JK, Hartung JE, Duque MG, Friedman R, Nagarajan V, Loeza-Alcocer E, et al. Distribution of functional opioid receptors in human dorsal root ganglion neurons. *Pain.* (2020) 161:1636–49. doi: 10.1097/j.pain.0000000000001846

32. Yaksh TL, Woller SA, Ramachandran R, Sorkin LS. The search for novel analgesics: targets and mechanisms. *F1000Prime Rep.* (2015) 7:56. doi: 10.12703/P7-56

33. Hartrick CT. Modeling neuropathic pain: subtlety and susceptibility. *Pain Pract.* (2011) 11:107–8. doi: 10.1111/j.1533-2500.2011.00452.x

34. Abboud C, Duveau A, Bouali-Benazzouz R, Massé K, Mattar J, Brochoire L, et al. Animal models of pain: diversity and benefits. *J Neurosci Methods.* (2021) 348:108997. doi: 10.1016/j.jneumeth.2020.108997

35. Chacur M, Milligan ED, Gazda LS, Armstrong C, Wang H, Tracey KJ, et al. A new model of sciatic inflammatory neuritis (SIN): induction of unilateral and bilateral mechanical allodynia following acute unilateral peri-sciatic immune activation in rats. *Pain.* (2001) 94:231–44. doi: 10.1016/S0304-3959(01)00354-2

36. Zitron I, Hartrick CT. Sciatic Inflammatory Neuritis: a non-surgical technique. *J Neuropathic Pain Symptom Palliation.* (2005) 1:3–12. doi: 10.3109/J426v01n04_02

37. Hartrick CT, Wise JJ, Patterson JS. Preemptive intrathecal ketamine delays mechanical hyperalgesia in the neuropathic rat. *Anesthesia Analgesia.* (1998) 86:557–60. doi: 10.1097/00000539-199803000-00022

38. Dickenson AH, Patel R. Translational issues in precision medicine in neuropathic pain. *Can J Pain.* (2020) 4:30–8. doi: 10.1080/24740527.2020.1720502

39. Niv D, Devor M. Chronic pain as a disease in its own right. *Pain Pract.* (2004) 4:179–81. doi: 10.1111/j.1533-2500.2004.04301.x

40. Treede R-D, Rief W, Barke A, Aziz Q, Bennett MI, Benoliel R, et al. Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). *Pain.* (2019) 160:19–27. doi: 10.1097/j.pain.0000000000001384

41. Yamamoto J, Kawamata T, Niiyama Y, Omote K, Namiki A. Down-regulation of mu opioid receptor expression within distinct subpopulations of dorsal root ganglion neurons in a murine model of bone cancer pain. *Neuroscience.* (2008) 151:843–53. doi: 10.1016/j.neuroscience.2007.11.025

42. Mantyh P. Bone cancer pain: causes, consequences, and therapeutic opportunities. *Pain.* (2013) 154:S54–62. doi: 10.1016/j.pain.2013.07.044

43. Harris RE, Clauw DJ, Scott DJ, McLean SA, Gracely RH, Zubieta JK. Decreased central mu-opioid receptor availability in fibromyalgia. *J Neurosci.* (2007) 27:10000–6. doi: 10.1523/JNEUROSCI.2849-07.2007

44. Bee LA, Bannister K, Rahman W, Dickenson AH. Mu-opioid and noradrenergic α(2)-adrenoceptor contributions to the effects of tapentadol on spinal electrophysiological measures of nociception in nerve-injured rats. *Pain.* (2011) 152:131-9. doi: 10.1016/j.pain.2010.10.004

45. Krebs EE, Gravely A, Nugent S, Jensen AC, DeRonne B, Goldsmith ES, et al. Effect of opioid vs nonopioid medications on pain-related function in patients with chronic back pain or hip or knee osteoarthritis pain: the SPACE randomized clinical trial. *JAMA.* (2018) 319:872–82. doi: 10.1001/jama.2018.0899

46. Ostling PS, Davidson KS, Anyama BO, Helander EM, Wyche MQ, Kaye AD. America's opioid epidemic: a comprehensive review and look into the rising crisis. *Curr Pain Headache Rep.* (2018) 22:32. doi: 10.1007/s11916-018-0685-5

47. Schäfer M, Imai Y, Uhl GR, Stein C. Inflammation enhances peripheral mu-opioid receptor-mediated analgesia, but not mu-opioid

receptor transcription in dorsal root ganglia. *J Pharmacol.* (1995) 279:165–9. doi: 10.1016/0014-2999(95)00150-J

48. Pol O, Alameda F, Puig MM. Inflammation enhances mu-opioid receptor transcription and expression in mice intestine. *Mol Pharmacol.* (2001) 60:894–9. doi: 10.1124/mol.60.5.894

49. Merighi S, Gessi S, Varani K, Fazzi D, Stefanelli A, Borea PA. Morphine mediates a proinflammatory phenotype via μ-opioid receptor-PKCε-Akt-ERK1/2 signaling pathway in activated microglial cells. *Biochem Pharmacol.* (2013) 86:487–96. doi: 10.1016/j.bcp.2013.05.027

50. Silva LCR, Castor MGME, Navarro LC, Romero TRL, Duarte IDG. κ-Opioid receptor participates of NSAIDs peripheral antinociception. *Neurosci Lett.* (2016) 622:6–9. doi: 10.1016/j.neulet.2016.04.029

51. Arendt-Nielsen L, Olesen AE, Staahl C, Menzaghi F, Kell S, Wong GY, et al. Analgesic efficacy of peripheral kappa-opioid receptor agonist CR665 compared to oxycodone in a multi-modal, multi-tissue experimental human pain model: selective effect on visceral pain. *Anesthesiology.* (2009) 111:616–24. doi: 10.1097/ALN.0b013e3181af6356

52. Brackley AD, Gomez R, Akopian AN, Henry MA, Jeske NA. GRK2 constitutively governs peripheral delta opioid receptor activity. *Cell Rep.* (2016) 16:2686–98. doi: 10.1016/j.celrep.2016.07.084

53. Berg KA, Rowan MP, Gupta A, Sanchez TA, Silva M, Gomes I, et al. Allosteric interactions between δ and κ opioid receptors in peripheral sensory neurons. *Mol Pharmacol.* (2012) 81:264–72. doi: 10.1124/mol.111.072702

54. Perl ER. Causalgia, pathological pain, and adrenergic receptors. *Proc Natl Acad Sci USA.* (1999) 96:7664–7. doi: 10.1073/pnas.96.14.7664

55. Hartrick CT. Noradrenergic reuptake inhibition in the treatment of pain. *Expert Opin Investig Drugs.* (2012) 21:1827–34. doi: 10.1517/13543784.2012.731393

56. Schroder W, Tzschentke TM, Terlinden R, De Vry RJ, Jahnel U, Christoph T, et al. Synergistic Interaction between the Two Mechanisms of Action of Tapentadol in Analgesia. *J Pharmacol Exp Ther (PET).* (2011) 337:312–20. doi: 10.1124/jpet.110.175042

57. Hartrick CT, Rozek RJ. Tapentadol in pain management: a μ-opioid receptor agonist and noradrenaline reuptake inhibitor. *CNS Drugs.* (2011) 25:359–70. doi: 10.2165/11589080-000000000-00000

58. *Nucynta ER (tapentadol) extended-release tablets for oral use C-II [Label].* (2008). Available online at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/200533s023lbl.pdf (accessed March 01, 2022).

59. Hartrick CT, Poulin D, Molenaar R, Hartrick A. Dual-acting peripherally restricted delta/kappa opioid (CAV1001) produces antinociception in animal models of sub-acute and chronic pain. *J Pain Res.* (2020) 13:2461–74. doi: 10.2147/JPR.S262303

60. Angst MS, Clark JD, Carvalho B, Tingle M, Schmelz M, Yeomans DC. Cytokine profile in human skin in response to experimental inflammation, noxious stimulation, and administration of a COX-inhibitor: a microdialysis study. *Pain.* (2008) 139:15–27. doi: 10.1016/j.pain.2008.02.028

61. Gustorff B, Hoechtl K, Sycha T, Felouzis E, Lehr S, Kress HG. The effects of remifentanil and gabapentin on hyperalgesia in a new extended inflammatory skin pain model in healthy volunteers. *Anesth Analg.* (2004) 98:401–7. doi: 10.1213/01.ANE.0000095150.76735.5D

62. Bishop T, Hewson DW, Yip RK, Fahey MS, Dawbarn D, Young AR, et al. Characterisation of ultraviolet-B-induced inflammation as a model of hyperalgesia in the rat. *Pain.* (2007) 76: 317–25.