Current Pain and Headache Reports (2021) 25: 29
https://doi.org/10.1007/s11916-021-00949-0

ALTERNATIVE TREATMENTS FOR PAIN MEDICINE (M JONES, SECTION EDITOR)

Dual Enkephalinase Inhibitors and Their Role in Chronic Pain Management

Warren A. Southerland[1] · Justin Gillis[1] · Sumanth Kuppalli[1] · Alex Fonseca[1] · Andrew Mendelson[2] · Storm V. Horine[2] · Nitin Bansal[2] · Amitabh Gulati[3]

Accepted: 12 February 2021 / Published online: 24 March 2021

Abstract

Purpose of Review Dual enkephalinase inhibitors (DENKIs) are pain medications that indirectly activate opioid receptors and can be used as an alternative to traditional opioids. Understanding the physiology of enkephalins and their inhibitors and the pharmacology of these drugs will allow for proper clinical application for chronic pain patients in the future.

Recent Findings DENKIs can be used as an alternative mode of analgesia for patients suffering from chronic pain by preventing the degradation of endogenous opioid ligands. By inhibiting the two major enkephalin-degrading enzymes (neprilysin and aminopeptidase N), DENKIs can provide analgesia with less adverse effects than nonendogenous opioids.

Summary The purpose of this paper is to review the current literature investigating DENKIs and explore their contribution to chronic pain management.

Keywords Enkephalinase inhibitors · Chronic pain · Multimodal analgesia · Pain management · Endogenous opioid · Analgesia

Introduction

Opioid use for acute and cancer pain is accepted and unmatched, but their role in chronic nonmalignant pain is unfounded, showing insignificant reductions in pain scores and limited improvement in quality of life [1•, 2, 3]. Though opioid agonists can provide effective peripheral, neuraxial, and systemic pain relief, adverse side effects, such as respiratory depression, sedation, nausea, and constipation, complicate patient care [1•, 4]. In addition, addiction to opioid agonists is common and plays a large role in the drugs' misuse, leading to overdoses and death [2, 5–7]. Misuse of opioids and poor management of chronic pain continues to be a major issue in medicine and public health, especially during the current opioid epidemic [2, 4–7]. In order to combat the opioid crisis, use of a multidisciplinary approach is required to treat chronic pain, utilizing multimodal analgesia with various medications of differing mechanisms along with nonpharmacological treatments (such as psychotherapy and physical therapy) [2, 4, 6, 8].

Dual enkephalinase inhibitors (DENKIs) are pain medications that indirectly activate opioid receptors and can be used as an alternative to traditional opioids. This class of drug has been shown to provide analgesia and, potentially, can help patients suffering from chronic pain. By inhibiting the two major enkephalin-degrading enzymes (neprilysin and aminopeptidase N) and enhancing endogenous opioids, DENKIs can provide analgesia with less adverse effects than nonendogenous opioids [9••, 10••]. This review discusses recent findings in this drug class and its future use as an alternative to opioids in chronic pain management.

Physiology of Enkephalinases

The primary pain control mechanism in mammals involve the opioid receptors and their associated ligands [11]. The three

This article is part of the Topical Collection on *Alternative Treatments for Pain Medicine*

✉ Warren A. Southerland
wsouther@bidmc.harvard.edu

[1] Beth Israel Deaconess Medical Center, Department of Anesthesia, Critical Care, and Pain Medicine, Harvard Medical School, Boston, MA, USA

[2] Department of Anesthesiology, NewYork-Presbyterian Hospital/ Weill Cornell Medical College, New York, NY, USA

[3] Department of Anesthesiology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

types of opioid receptors are mu, delta, and kappa, which are G-protein-coupled receptors expressed by central and peripheral neurons [1•, 12, 13]. These receptors influence the excitability of neurons, reducing the release of pronociceptive and proinflammatory neuropeptides [14–16]. Opioid medications, such as morphine and oxycodone, provide analgesia by imitating endogenous ligands at these receptors [17, 18].

Enkephalins are endogenous opioids, first discovered in 1975, and remain an active area of research and pharmaceutical development. The enkephalins are one of the three peptide systems that also include beta-endorphins and dynorphins. Of note, the three classes of endogenous opioid peptides share a common N terminus sequence of Tyr-Gly-Gly-Phe and lack a C terminus amide [19•]. The enkephalin class of molecules are pentapeptides that are characterized into two subgroups by the presence of methionine or leucine at their carboxy-terminal amino acids. Thus, enkephalins are classified either as met-enkephalins (Tyr-Gly-Gly-Phe-Met) or leu-enkephalins (Tyr-Gly-Gly-Phe-Leu), respectively [20]. The enkephalin molecule is generated through the cleavage of a precursor molecule known as pro-enkephalin, creating either met-enkephalin or leu-enkephalin.

Enkephalins contribute to endogenous pain regulation, which is proven by elevated sensitivity to noxious stimulation after inhibition of enkephalin synthesis [21, 22]. These peptides act as neurotransmitters and neuromodulators throughout the nervous system and various end-organ targets [11]. Enkephalins exert their physiological effect through specific opioid receptors: mu (largely expressed in the central nervous system), delta (in the substantia nigra pars compacta and spinal cord), and kappa (expressed primarily in the spinal cord) [20]. The physiological effects include, but are not limited to, its role in analgesia, angiogenesis, blood pressure regulation, hypoxia, memory processes, neuroprotection, pancreatic secretion, wound repair, respiratory control, and hepatoprotective mechanisms [19•] (see Table 1).

Enkephalins undergo biodegradation via hydrolysis, which cleaves the pentapeptide at the Tyr-Gly bond. Enkephalinases and aminopeptidases further degrade the molecules into shorter peptides that are from 2 to 4 amino acids in length [19•]. It was initially noted to be difficult to differentiate distinct enkephalin-degrading enzymes (so called "enkephalinases") from other enzymes in the central nervous system (CNS) [23]. Eventually, researchers identified a specific enkephalin-degrading peptidase with a regional distribution pattern that suggested localization in the same vicinity as opioid receptors in the brain [24]. Further research on these enkephalinase molecules found that enkephalins are degraded by several membrane-associated brain peptidases. Two carboxy-directed dipeptidyl peptidases (named enkephalinase A1 and A2) degrade synaptically released Met- and Leu-enkephalin to the Tyr–Gly–Gly fragment [25]. An amino-terminal-directed dipeptidylpeptidase ("enkephalinase B") degrades the enkephalins to Tyr–Gly [25]. Subsequently, it was found that the "enkephalinase" neprilysin is an endopeptidase that cleaves at the Gly^3–Phe^4 bond and is specific for the enkephalinergic system [26, 27].

Through continued experimentation, multiple approaches to enkephalin degradation were explored. Baume et al. in 1983 demonstrated various methods of metabolism of enkephalins through inhibition of either the enkephalinase, the aminopeptidase, or both molecules [28]. Their team found that inhibiting the singular enkephalinase, or aminopeptidase, would partly inhibit the metabolism of Met-enkephalin [28]. Furthermore, their combined inhibition would almost entirely prevent the metabolism of this molecule [28]. In terms of clinic effects, the researchers witnessed that inhibition of the enzymes resulted in an analgesic effect which could be blocked by administration of naloxone [29]. Studies have found that inhibitors of only a single enzymatic pathway of enkephalin degradation do not produce a significant analgesic effect; rather, both pathways must be blocked in order to produce a marked antinociceptive effect [10••]. Hence, it is inhibition of both degradative enzymes, as occurs by DENKIs, that exerts an antinociceptive effect by prolonging the action of endogenous enkephalins [9••].

Table 1 Biophysiological mechanisms of enkephalins (modified from Duque-Díaz et al. [19•]

Analgesia
Angiogenesis
Blood pressure regulation
Hypoxia
Memory processes
Neuroprotection
Pancreatic secretion
Wound repair
Respiratory control
Hepatoprotective mechanisms

Pharmacology of Enkephalinase Inhibitors

Early enkephalinase inhibitors were used to study the characteristics of enkephalinases in the 1980s. These molecules included puromycin, which inhibits aminopeptidase, and Gly-Gly-Phe-Met, which inhibits neprilysin (enkephalinase) [24, 30]. It was found that the inhibitory activity of these molecules involved an aromatic moiety (to interact with a hydrophobic region of the enzyme) and a terminal carboxy group on a small amino acid [31]. Since the discovery of enkephalinase inhibitors and their ability to provide antinociceptive effects in animals (equivalent to analgesia in humans) by amplifying the action of endogenous opioids, researchers began exploring potent and selective molecules for enkephalinase inhibition

Table 2 Two dual enkephalinase inhibitors (DENKIs) currently under investigation (modified from Raffa et al. [10••], Bonnard et al. [40], and Roques et al. [41])

PL37	PL265
• First oral DENKI, developed as a prodrug with excellent bioavailability • Forms two active metabolites that peripherally inhibit NEP and APN • For treatment of postoperative pain • Currently in phase I clinical trials (started in 2019)	• Focuses on peripheral action • Forms one metabolite that blocks both NEP and APN • For treatment of diabetic neuropathy • Started clinical trials in 2018

[31–37]. In addition, the additive analgesic effect of blocking both enzymes responsible for enkephalin metabolism lead to the development of dual enkephalinase inhibitors.

Research showed that kelatorphan, an early DENKI, caused antinociceptive effects in mice that were greater than the single enzyme inhibitors combined [38]. Examples of other inhibitors that showed antinociceptive effects in animal studies include PC12, RB101, RB120, RB3007, PL37, and PL265 [39–41]. In order to provide more systemic distribution, a prodrug version of DENKIs, RB101, was synthesized by combining neutral endopeptidase inhibitors by a disulfide or thioester bond [42]. One study has shown that this prodrug provides antinociceptive effects in mice and rats, which can be suppressed by naloxone [43]. Different DENKIs exhibit various distributions throughout the body, with differences in their ability to cross the blood-brain barrier and enter the central nervous system [9••]. For example, RB101, RB120, and PL37 (at high doses) can enter the brain, while kelatorphan and PL265 cannot [9••].

Since enkephalins are present at peripheral nerves sites and more than half of the analgesic effects of exogenous opioids occur in the periphery, these drugs do not have to pass the blood-brain barrier to be effective [44–49]. Studies focusing on the aforementioned kelatorphan and RB101 describe antinociceptive effects in various animal models from their peripheral action [50, 51]. Because of these findings, research has focused on DENKIs that act peripherally to inhibit pain signals and have limited effect in the brain in order to reduce opioid-related side effects [10••].

Uses in Chronic Pain Management

Opioids have been improperly used to treat chronic nonmalignant pain, contributing to the current opioid epidemic [2, 4–7]. Though they are effective analgesics, their side effect profile, which includes respiratory depression, sedation, nausea, and constipation, creates obstacles for pain management [1•, 4]. Addiction to opioids contributes to the drugs' misuse, leading to overdoses and death [2, 5–7]. Alternative modes of analgesia have been the focus of current research, including the enhancement of endogenous opioids.

When compared to endogenous opioids, such as enkephalins, exogenous opioids (1) do not have same distribution and relative concentration at active sites and (2) are not well controlled at the synaptic level, leading to poor regulation of the drug's effects and frequent overdosing [10••]. Endogenous opioids are released in areas of the body that require pain relief while opioid agonists are distributed throughout the entire body, regardless of their need. Due to this systemic distribution, high concentrations of opioid drugs are available at unessential sites, including the CNS, and disrupt the local homeostasis at these sites. To avoid this direct activation of opioid receptors by exogenous agonists, enhancement of endogenous opioids by indirect means, such as enkephalinase inhibitors, exerts their analgesic effects by protecting endogenous enkephalins from enzymatic degradation [29]. This concept prevents liberally distributed opioid agonists from causing adverse side effects, desensitization, and tolerance [1].

A major proposed clinical advantage of DENKIs is reduced opioid adverse effects such as respiratory depression, sedation, and addiction [1•]. Endogenous opioids such as enkephalins are released locally to in-demand areas and have a high affinity to opioid receptors, leading to decreased systemic opioid activity [10••]. This is particularly beneficial for those who are more prone to these adverse effects, such as the elderly, children, and individuals at higher risk for addiction. DENKIs do not need to cross the blood-brain barrier to produce analgesia, and recently focus has shifted to blocking noxious stimuli from entering the central nervous system [10••, 45–47]. Not only does this reduce the centrally acting adverse effects, but pain processes with specific peripheral action can be targeted, such as inflammatory and neuropathic pain [10••, 50, 52, 53]. Additionally, with DENKIs' minimal activity in the CNS, abuse of the drugs is less likely.

Current animal and human studies continue to show that inhibition of both endopeptidase neprilysin (NEP) and the aminopeptidase N (APN), not just a single enzymatic pathway, is required for clinically significant analgesia [10••, 52, 54]. Pharmaceutical studies have shown significant promise for these dual enkephalinase inhibitors [55]. The antinociceptive effect of enkephalinase inhibitors has led to the exploration of its clinical utility in pain management. Its potent analgesic qualities have been investigated in humans

previously, such as a 1988 study on cancer patients previously unresponsive to morphine who received intrathecal administration of an enkephalinase inhibitor, resulting in significant, long-acting analgesia [56]. Currently, two DENKIs are in trials for their use for postoperative pain and neuropathic pain.

Additionally, synergistic effects have been seen when administering DENKIs with morphine, cannabinoid, purinergic receptor (P2X3) antagonists, and cholecystokinin (CCK) antagonists [57–60]. Clinically, this could help improve pain management, beyond that of DENKIs alone, and lessen the adverse effects from larger doses of opioids and other analgesics.

Future Considerations

There are two pharmaceutical companies with DENKIs that are currently in clinical studies for neuropathic and postoperative pain [9••] (see Table 2). One example is PL37, a DENKI that is currently in phase I clinical trials (started in 2019) and is being evaluated for postoperative pain [10••]. This formulation is the first oral DENKI, developed as a prodrug with excellent bioavailability. PL37 forms two active metabolites that peripherally inhibit NEP and APN [10••, 41]. Another example is PL265, which started clinical trials for treatment of diabetic neuropathy in 2018. The design of this medication focuses more on concentrated peripheral action with just one metabolite that blocks both NEP and APN [10••, 40]. STR-324 is an analog to opionorphine, a specific DENKI found in human saliva, that has performed well in postoperative and neuropathic pain models with limited opioid adverse effects [61, 62•]. Enkephalinase inhibition is an area of pharmacology with limited human studies; therefore, it is prudent to be aware of the long-term consequences of NEP and APN inhibition since they are involved in the metabolism of several other bioactive peptides [63]. Future research to analyze this drug class's effects in humans is possible since the FDA approved the first dual-acting angiotensin-receptor–NEP inhibitor for the treatment of heart failure in 2015 [9••].

Conclusions

Many chronic pain patients have been inappropriately prescribed opioids, leading to overuse and addiction. Additionally, due to the various side effects from opioid medications, long-term management of pain can be complicated by respiratory depression, sedation, nausea, and constipation. Due to the current opioid crisis, an alternative mode of analgesia is essential, especially one with less adverse effects and a better safety profile. Dual enkephalinase inhibitors provide a more natural treatment of pain by utilizing the body's own pain modulators and enhancing their effect by decreasing their degradation. Currently, two DENKIs for neuropathic and postoperative pain are undergoing clinical trials. Future studies are required to further evaluate the drugs' effectiveness and any adverse effects from inhibiting these enzymes.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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