The novel, non-opiate Dual Enkephalinase Inhibitors (DENKI) provide Safe and Effective Pain Relief

PST524

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Introduction

Aim of investigation:

Painkillers with novel mechanisms of action are being sought as alternatives to opioids and non-opioids/NSAIDS. Innovative non-opiate and non-addictive products were developed for the management of acute and chronic severe pain, a growing market with significant unmet medical need. These new drugs leverage the power of endogenous enkephalins, a key element of the body's natural pain modulation system. **Dual ENKephalinase inhibitors (DENKIs**) aim to protect enkephalins from their rapid enzymatic degradation, hence increasing their local concentrations. The resulting physiological analgesia provides a safe and highly effective new pain management option.

DENKI's mechanism of action

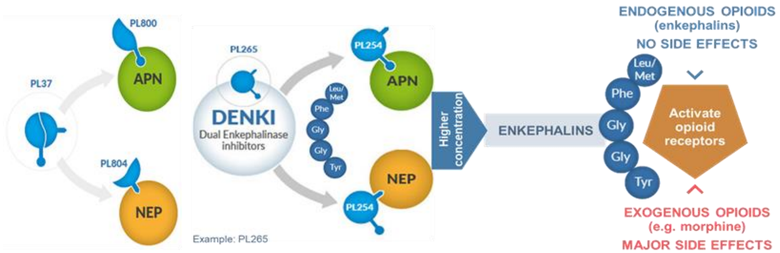
Enkephalins are the most abundant endogenous opiates; they are widely expressed and bind both δ and μ opioid receptors. They induce analgesia centrally and peripherally by inhibiting pain signaling.

Peripherally, they suppress tetrodoxin-resistant Na^+, TRPV1 and other cation currents stimulated by inflammatory agents. Through these effects, they attenuate peripheral nociceptor terminal excitability, the propagation of action potentials and the release of excitatory pro-inflammatory neuropeptides (SP, CGRP).

Once released, the opioid peptides Met-enkephalin, YGGFM, and Leu-enkephalin, YGGFL, are inactivated by two zinc-metallopeptidases, NEP (EC 3.4.24.11) and aminopeptidase N, APN (EC 3.4.11.2) providing the opportunity to design enzyme inhibitors as **new efficacious analgesics** acting selectively when and where enkephalins are released after noxious stimulus.

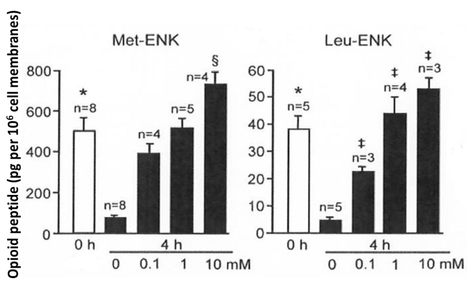
However, inhibiting only one of these enzymes does not increase enkephalin concentrations enough to induce **analgesic responses.**[1] We have therefore developed the concept of "dual inhibitors" which **inhibit both NEP and APN[2].** Accordingly, several families of dual inhibitors were designed and synthetized[2, 3, 4] and shown to be very active by various administration routes.

Two Pharmaleads' Dual ENKephalinase Inhibitors (DENKI), **PL37** and **PL265**, are now in clinical development as new analgesics devoid of the side effects of morphine and derivatives. Both are active *per os* without entering the CNS while i.v. PL37 crosses the blood brain when properly formulated. Their efficacy was assessed in a wide array of pain models and their safety/tolerability were evaluated, in particular with regards to opioid-like side-effects.



DENKIs protect Enkephalins from degradation

A single dose of aminophosphinic dose-dependently prevents the degradation of ENK on peripheral nerves from inflamed paws



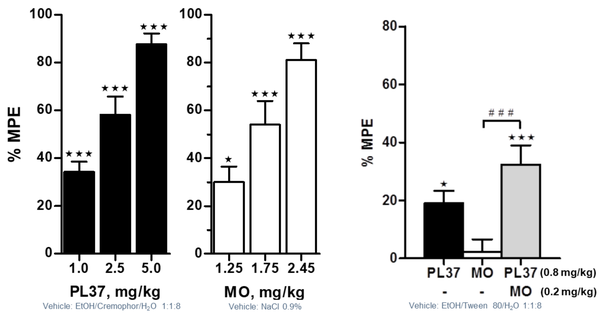
Radioimmunoassays showing that inhibition of leukocytic APN and NEP by an aminophosphinic DENKI prevents the degradation of enkephalins, secreted in rats' hindpaw after intraplantar administration of complete Freund's adjuvant (150μL). Experiments were performed 4 days after triggering inflammation[5].

Methods

The antinociceptive properties of DENKIs (PL37 and PL265) administered through different routes were evaluated using various animal pain models (acute, chronic, neuropathic, cancer).

Experiments were performed in male OF1 mice (Charles River Laboratories, France), and male Sprague−Dawley rats (Janvier, France). Animals were housed for at least 2 days before experiments in a room with controlled temperature (21 ± 2 °C) and a 12 h alternating light−dark cycle. Food and water were available at libitum. Great care was taken to avoid or minimize discomfort of animals. Animal experiments were carried out in accordance with the European Communities Council directive (89/609/CEE) and with the ethical guidelines of the International Association of Pain. The maximal volume administered was 0.1 mL/100g for intravenous injection and 0.2 mL/100g orally.

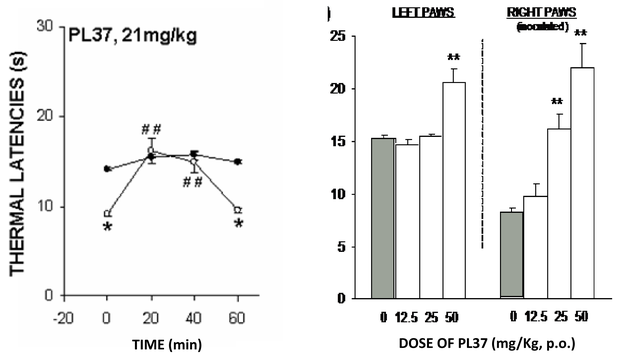
iv PL37: acute central pain – HPT in mice



Both PL37 and morphine (MO), induced dose-dependent antinociceptive responses with comparable ED_{50}s of 1.8 (1.2-2.6) and 1.6 (1.4-2.0) mg/kg for PL37 in EtOH/cremophor/water and MO in saline respectively (left side).

A significant increase in antinociceptive response was observed by co-administration of inactive or sub-optimal doses of PL37 (0.8 mg/kg) and MO (0.2 mg/kg) by i.v. route (right side), suggesting a **synergistic effect.**

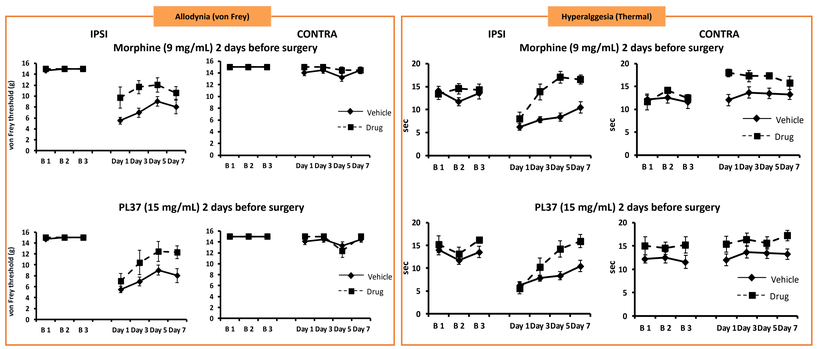
Oral PL37: Cancer Pain – tibial osteosarcoma in mice



The antinociceptive effect of PL37 was studied using the validated animal model of tibial osteosarcoma. In this model, murine sarcoma cells are injected into the mouse tibia after which the animal develops severe thermal hyperalgesia and allodynic symptoms[6].

Oral administration of **PL37 (25 mg/kg) inhibited osteosarcoma-induced thermal hyperalgesia measured by the unilateral hot-plate test with a maximal effect observed between 20 and 40 minutes after administration (left).** The dose-response histogram displayed on the right measures the effect of increasing doses of oral PL37 on thermal hyperalgesia 20 minutes after administration and shows that there was **complete inhibition of hyperalgesia at the dose of 25 mg/kg.**

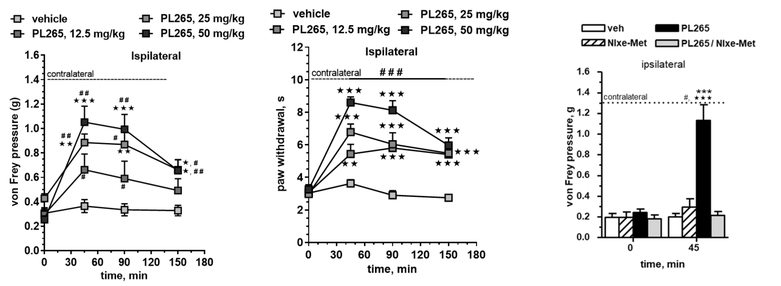
iv PL37: acute post operative pain – Incisional Pain



The antinociceptive effects of a 7 day continuous iv infusion of PL37 on the development of incisional pain in rats were investigated and compared to those of morphine. Incisional pain was developed after a 1-cm longitudinal incision in the plantar aspect of the right hind paw[7]. Morphine or PL37 were administered iv 2 2 days before and 5 days after surgery.

Both allodynia (von Frey, left panels) and thermal-induced hyperalgesia (right panels) were reversed by morphine (top panels) and PL37 (bottom panels).

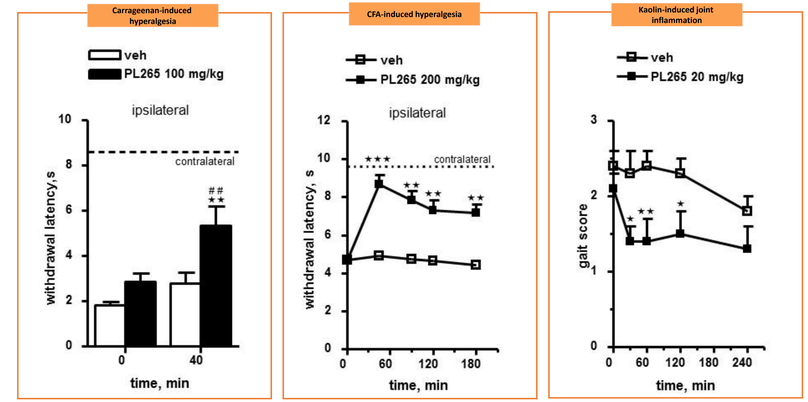
Oral PL265: neuropathic pain model – PSNL in mice



Single oral administration of PL265 in mice produced dose-dependent **antiallodynic (left panel) and hyperalgesic (middle panel) effects lasting at least 150 min[4].**

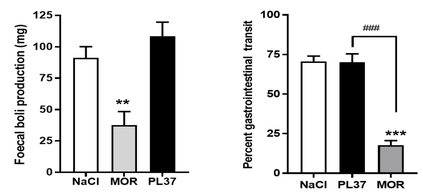
The **antiallodynic effect** at PL265 50 mg/kg was **fully reversed by co-injection of naloxone methiodide (5 mg/kg, i.p.), indicating that only peripheral opioid receptors are recruited (right histogram).**

Oral PL265: inflammatory pain models



PL265 *per os* significantly increased paw withdrawal thresholds (thermal stimulus)	PL265 *per os* significantly increased paw withdrawal thresholds (thermal stimulus) and displayed a long-lasting effect	PL265 (i.v.) effectively reduced gait score for 120 minutes.

Lack of GI effects



Unlike morphine (10 mg/kg s.c.) which produces a significant decrease in faeces production (left) and in transit time (right), PL37 (80 mg/kg s.c.) does not induce any of these G.I. side-effects.

Conclusions

Across a wide array of animal models of pain, DENKIs induce antinociceptive responses similar to morphine. DENKIs remain active after chronic administration of morphine, indicating that DENKI-induced analgesia persists in morphine-tolerant mice. Moreover, DENKIs do not induce constipation in animals or humans. Finally, PL37 and PL265 were both shown as devoid of any abuse liability even at high doses and after repeated administration (not shown) and could be used as a new class of painkillers in all types of severe pain as an affordable solution to the opioid crisis.

Based on these remarkable pharmacological results, DENKIs are currently in clinical development as novel painkillers. PL265 is being developed for the oral treatment of neuropathic pain, inflammatory diseases of the bowel, osteoarthritis pain or topic treatment of ocular / inflammatory pain whereas PL37 targets the treatment of post-surgical / traumatic / breakthrough cancer pain as a substitute to injectable opiates.

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