Dual enkephalinase inhibitor (DENKI) PL265: a novel topical treatment for ocular pain ?

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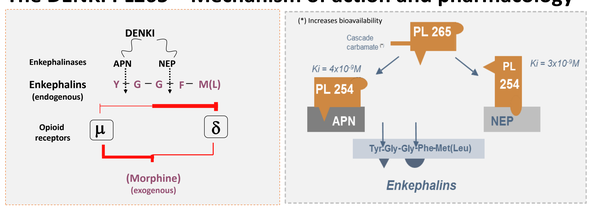
INTRODUCTION

The peripheral endogenous opioid system is critically involved in neuropathic and inflammatory pain (1). Enkephalins, the main endogenous opioids, play a key role at all levels of pain control and exhibit an analgesic efficacy comparable to morphine without the adverse effects.

Dual **ENK**ephalinases **I**nhibitors (DENKIs) are specific and selective inhibitors of aminopeptidase N (APN) and neprilysin (NEP), protecting enkephalins from enzyme degradation. DENKIs, thereby potentiate physiological functions of enkephalins (e.g. pain control).

The DENKI PL265 – Mechanism of action and pharmacology

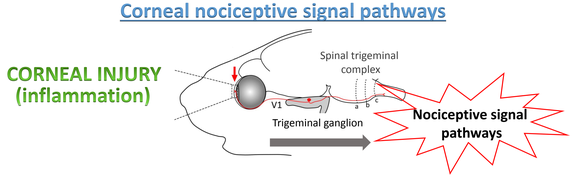


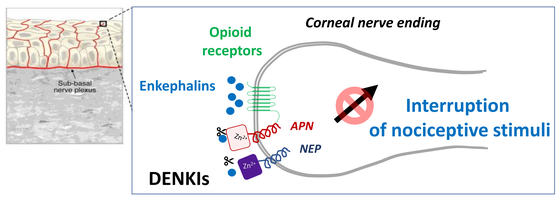
- PL265 is a small non-peptide molecule (MW: 576 Da).
- PL265 is active orally in all rodent models of neuropathic pain tested with a strictly peripheral effect (2).
- PL265 has been given orally to rats (up to 1200 mg/kg) and dogs up to 800 mg/kg) without toxicity.
- Oral PL265 has safely completed Phase 1 single ascending dose (100-800 mg) and is now in Phase 1 multiple ascending dose (up to 300 mg b.i.d.) for 4 days, before entering Phase 2 in 2018 in painful diabetic neuropathy.

PURPOSE

- All elements of the enkephalinergic system (opioid receptors, enkephalins and their degrading enzymes) are also expressed in ocular surface tissues. Topical morphine has actually been shown to relieve pain associated with corneal lesions in rat and dog pain models (3,4).
- To date, no topical ocular analgesics are available for the treatment of acute or chronic pain, only anesthetics which have many shortcomings.
- Here, we display the antinociceptive and anti-inflammatory effects of a highly effective DENKI prodrug, PL265, using experimental murine models of ocular nociception and inflammation.

Corneal nociceptive signal pathways





METHODS

Adult male C57BL/6 mice (8-week old) were used. Animal procedures were carried out by authorized investigators in accordance with institutional guidelines for the care and use of experimental animals approved by the European Communities Council Directive 2010/63/UE).

Corneal injury model

Under anesthesia, a corneal scraping was performed in one eye with a 1.5 mm trephine (Beaver Visitec) at day 0 (D0). Non-operated and operated mice were treated twice a day either with a drop of PL265$(ONa)_2$ (10 mM) or with PBS (control animals) in the right eye for 5 days.

Inflammatory pain

After corneal injury, some operated animals received a drop of LPS (0111:B4, Sigma, 50 µg/10 µl). Non-operated and operated mice were treated twice a day either with a drop of PL265$(ONa)_2$ or with PBS (control animals) in the right eye for 5 days.

In a second set of experiments, some animals received a drop of naloxone methiodide (100 µM, Sigma), a non selective opioid receptor antagonist, which does not cross the blood brain barrier, 15 minutes before a topical instillation of PL265$(ONa)_2$ (10 mM).

The corneal integrity and inflammation were evaluated by *in vivo* confocal microscopy (IVCM, Heidelberg Retina Tomography (HRT)II/Rostock Cornea Module Heidelberg) at D5.

Behavioral tests

Mechanical and chemical corneal sensitivities were evaluated using von Frey filaments (Bioseb) and capsaicin (100 µM, Sigma) instillation at D5.

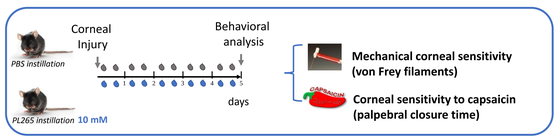
Von Frey filaments were applied onto the center of the cornea and the mechanical threshold was determined by blinking response **15 minutes after the last instillation**.

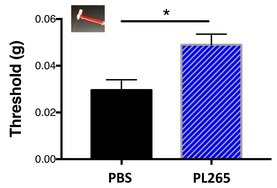
Data are presented as means ± SEM and analyzed using Prism™ software.

RESULTS

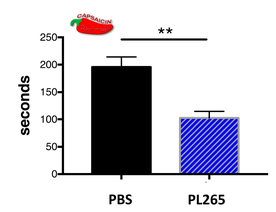
In non-operated mice (without corneal injury), topical instillation of PL265 for 5 days does not alter ocular surface (slit lamp and IVCM examinations) or corneal mechanical sensitivity compared to PBS-treated mice.

Corneal injury model



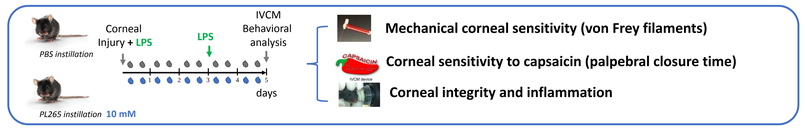


Topical instillation of PL265 (10 mM) significantly increased corneal mechanical threshold at D5 compared to PBS-treated mice (49.00 ± 4.58 mg *vs* 29.60 ± 4.43, n=10 per group).

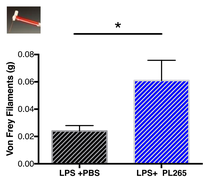


In addition, the palpebral closure time induced by topical capsaicin was significantly decreased (97 *vs* 210 seconds, n= 10 per group) after PL265 treatment.

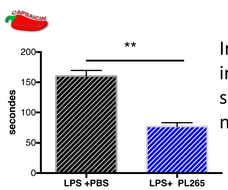
EFFECTS OF TOPICAL PL265 ON AN INFLAMMATORY PAIN MODEL



- **Topical PL265 elicits significant antinociceptive effects**

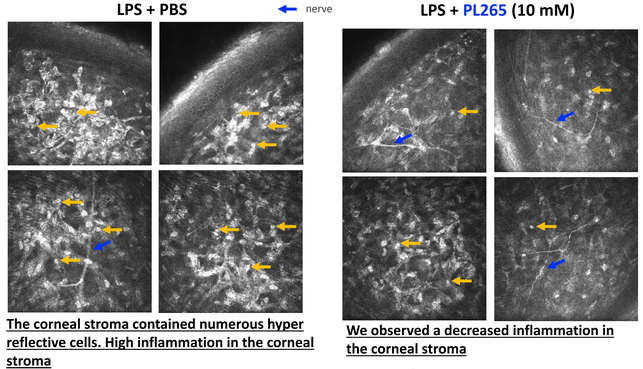


Topical PL265 shows potent antinociceptive effects. Mice treated with PL265 exhibited an increased mechanical threshold 61.00 ± 14.70 mg vs 24.00 ± 4.00 mg, n=5 at D5



In addition, the palpebral closure time induced by topical capsaicin (100 µM) was significantly decreased (70 *vs* 170 seconds, n= 5 per group) after PL265 treatment.

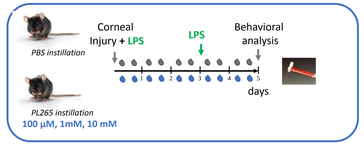
- **Topical PL265 decreases corneal inflammation**

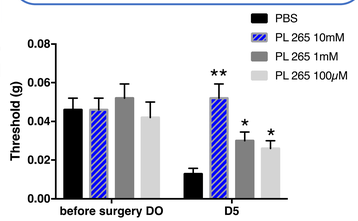


The corneal stroma contained numerous hyper reflective cells. High inflammation in the corneal stroma

We observed a decreased inflammation in the corneal stroma

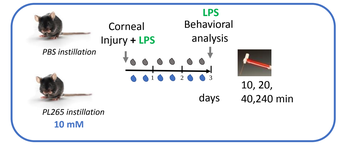
- **Dose-response of PL265**

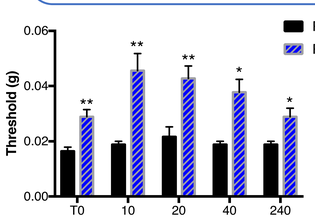




Dose response of PL265 eye-drops. This confirms that the 10 mM dose was relevant

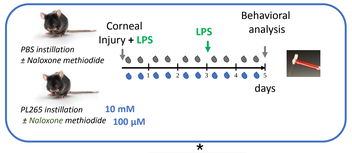
- **Time-course of action of PL265**

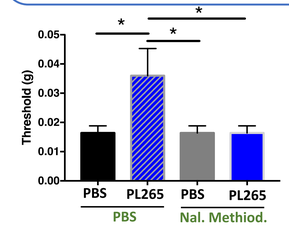




Time course of action of PL265(10 mM) eye-drops shows that the effects on LPS-induced inflammation last for at least 4 hours

- **Reversion of antinociceptive effects by naloxone methiodide**



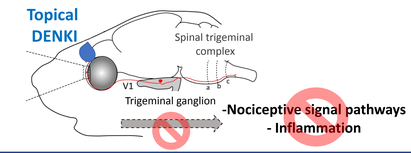


PL265 antinociceptive effects are purely peripheral and are mediated by the opioid receptors on the corneal surface

CONCLUSIONS

- This study provides the first evidence that PL265, a prodrug from a new therapeutic class, DENKI, is highly effective in **decreasing corneal nociception** after various experimental corneal lesions.
- PL265 also **significantly reduces corneal inflammation** in a model of LPS-induced inflammatory pain.
- The antinociceptive effects of PL265 are mediated by opioid receptors on ocular surface nerve endings, without involvement of the central nervous system.
- PL265 appears as **a promising topical medication for safely and effectively alleviating ocular pain and inflammation.**

How PL265-protected enkephalins block nociceptive signal pathways



References:

[1] Roques B.P., Fournie-Zaluski M.C., and Wurm M. *Inhibiting the breakdown of endogenous opioids and cannabinoids to alleviate pain*. Nature Rev. Drug Discov., 2012, 11, 292-310.

[2] Bonnard E., Poras H., Nadal X., Maldonado R., Fournié-Zaluski M.C., Roques B.P. *Long-lasting oral analgesic effects of N-protected aminophosphinic Dual ENKephalinase Inhibitors (DENKIs) in peripherally controlled pain*. Pharmacol. Res. Persp., 2015, 3(2), e00116, doi: 10.1002/prp2.116.

[3] Stiles J., Honda CN., Krohne SG., Kazacos EA. *Effect of topical administration of 1% morphine sulfate solution on signs of pain and corneal wound healing in dogs*. Am. J. Vet. Res., 2003, 64, 813-818.

[4] Wenk HN., Nannenga MN., Honda CN. *Effect of morphine sulfate eye drops on hyperalgesia in the rat cornea*. Pain, 2003, 105, 455-465.