Dual ENKephalinase Inhibitor (DENKI) PL37: A possible new class of migraine therapeutics

Amélie DESCHEEMAEKER[1], Hervé PORAS[2], Tanja OUIMET[2], Philippe LUCCARINI[1], Michel WURM[2], Radhouane DALLEL[1]

[1] Neuro-Dol – Inserm/UCA U1107, Faculté de Chirurgie Dentaire, 2 rue de Braga, 63100 Clermont-Ferrand, France; [2] Pharmaleads SA, 11 rue Watt, 75013 Paris, France

Introduction

Aim of investigation:

Migraine is a chronic neurovascular disorder with episodic manifestations (attacks) typically characterized by unilateral and pulsating severe headache, lasting 4 to 72 hours, accompanied by autonomic nervous system dysfunction and various neurological symptoms[1]. Clinical studies have identified risk factors for migraine transformation: one is cutaneous allodynia. Two-thirds of migraineurs exhibit allodynia during[2-4] but also between migraine attacks[5]. It is assumed that allodynia is a risk factor because it is a marker of central sensitization[3, 6-8].

Most opioid analgesics in common clinical use for migraine and other pain conditions primarily target the μ-opioid receptor. However, these μ-receptor agonists have relatively poor efficacy as analgesics for migraine headache, and can contribute to progression of migraine[9-10]. The main endogenous opioids endowed with antinociceptive properties are Met-enkephalin and Leu-enkephalin. They are expressed as pre-propeptides (preproenkephalin (PENK)) and interact specifically with two G protein-coupled receptors: the μ-opioid receptors (MORs) and the δ-opioid receptors (DORs). The affinity of enkephalins for MORs is similar to that of morphine, whereas their affinity for DORs is about tenfold higher[11]. It has been shown that the protection of extracellularly released endogenous opioid peptides (enkephalins) from their inactivation by peptidase inhibitors provide analgesia with reduced side effects comparatively to exogenous opioids[11].

PL37 belongs to a new pharmacological class of analgesics, the **D**ual **ENK**ephalinase **I**nhibitors (**DENKI®**), small molecules which protect enkephalins from their rapid degradation by the metalloenzymes neutral endopeptidase and aminopeptidase N, thereby increasing the duration of their analgesic action[11]. Its efficacy has been demonstrated in a wide range of rodent preclinical models of neuropathic, inflammatory and acute pain[12-13].

This study tested the antimigraine potential of PL37 in acute and chronic animal model of migraine.

Summary of pharmacological background

The efficacy of PL37 has been demonstrated in a wide range of rodent preclinical models of acute pain: HPT in mice, TFT in rats, acetic acid writhing test, Brennan incisional pain model in rats and of chronic pain: CCI in rats, PSNL in mice, streptozotocin-induced diabetic neuropathy in mice/rats, tibial osteosarcoma in mice, vincristine-induced neuropathy. Depending on the model and the animal species, efficacy appeared at acute doses between 1 and 50 mg/kg (published [12,13] and unpublished data).

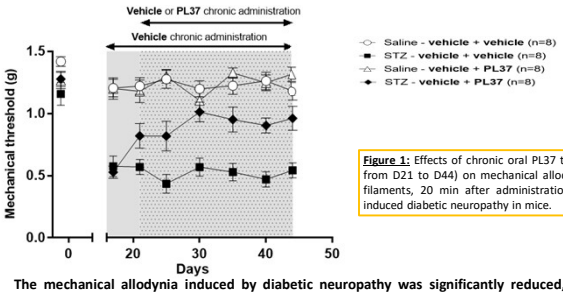


Figure 1: Effects of chronic oral PL37 treatment (20 mg/kg, bid from D21 to D44) on mechanical allodynia, measured with VF filaments, 20 min after administration, in streptozocin (STZ)-induced diabetic neuropathy in mice.

The mechanical allodynia induced by diabetic neuropathy was significantly reduced, from D21 to D44 of treatment.

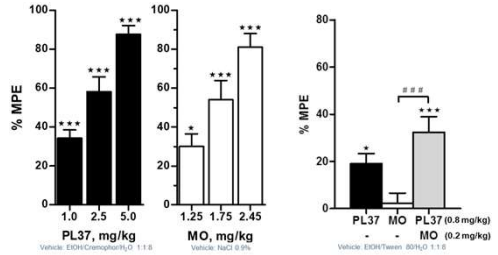


Figure 2:
(Left) Effects of iv PL37, morphine on hot plate test (HPT) in mice, a model of acute pain.
(Right) Effects of iv combination of sub-active dose of PL37 (0.8 mg/kg) with morphine (0.2 mg/kg) on hot plate test (HPT) in mice.

Both compounds induced dose-dependent, high antinociceptive responses with comparable ED_{50}s.
PL37 co-administered with morphine significantly potentiates its analgesic effects, suggesting a synergistic effect.

Acute migraine model – Oral administration

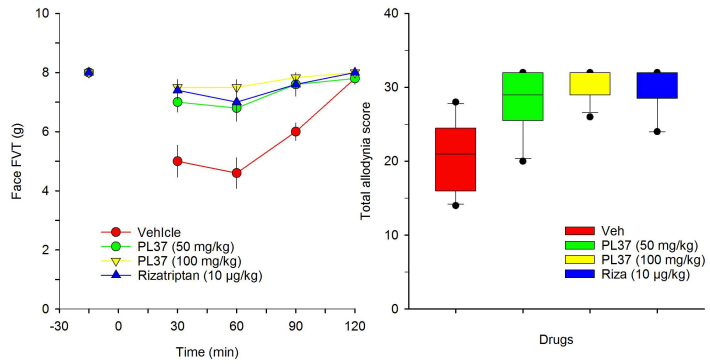
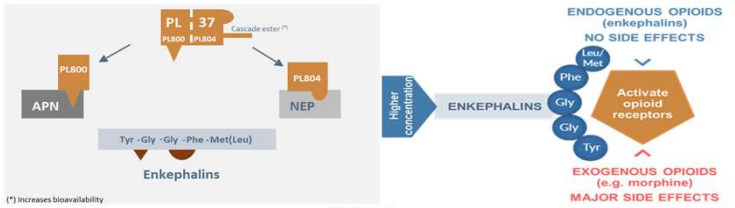


Figure 3: Effect of oral administration PL37 and rizatriptan on ISDN-induced cephalic mechanical allodynia. Time courses of von Frey withdrawal thresholds (VFT) of the face in saline (red circles, n = 10), PL37 (green circles, 50 mg/kg, n = 10; yellow triangles, 100 mg/kg, n = 10) and Rizatriptan treated (blue triangles, 10 μg/kg, n = 10) rats after a single ISDN injection. Values are means ± s.e.m. *Two-way ANOVA followed by Dunn's post hoc test, with (P < 0.001) and treatment (P < 0.001) as factors.*

Boxplots (median of the sum of the values over the analyzed period) showing the antimigraine effects of PL37 (50 mg/kg, po), PL37 (100 mg/kg, po) and rizatriptan (10 μg/kg, po) in rats treated by a single ISDN injection. *Kruskal-Wallis one-way ANOVA on ranks with post-hoc Dunn's test.*

Oral administration of PL37 at 50 and 100 mg/kg prevent cephalic mechanical allodynia induced by a single administration of isosorbide dinitrate (ISDN).

PL37 – DENKI's mechanism of action



Methods

Experiments were performed on male Sprague-Dawley CD rats according to the ethical guidelines of the International Association for the Study of Pain, the Directive 2010/63/UE of the European Parliament and the Council on the protection of animals used for scientific purpose.

The effects of intravenous, subcutaneous or oral administration of drugs (PL37 20 mg/kg, iv or 50 and 100 mg/kg, *per os*, sumatriptan 0.3 mg/kg, iv; rizatriptan 10 μg/kg, *per os,* naloxone-methiodide 5 mg/kg, sc) were tested on cephalic cutaneous mechanical sensitivity triggered by the acute or chronic dosing of a nitric oxide donor, isosorbide dinitrate (ISDN), a known migraine trigger[14].

The cephalic cutaneous mechanical sensitivity was assessed using von Frey filaments, calibrated to exert a constant force to determine the strength (threshold) which causes a withdrawal reaction of the head.

Chronic migraine model – Intravenous administration

Intravenous administration of PL37 (20 mg/kg) or sumatriptan significantly reduced persistent cephalic mechanical allodynia induced by recurrent administration of ISDN.
For both drugs, the anti-allodynic effects were significant 30 min after ISDN administration, and were significantly stronger for PL37 (at least for 4 h) than for sumatriptran.

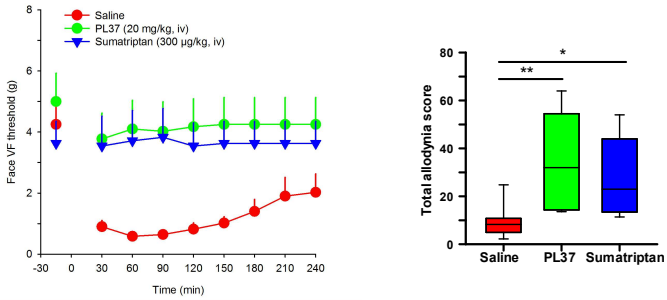


Figure 4: Effects of intravenous administration of PL37 and sumatriptan on ISDN-induced cephalic mechanical allodynia. Time courses of von Frey withdrawal thresholds (VFT) of the face in saline (black circles, n = 8), PL37 (red circles, 20 mg/kg, n = 8) and sumatriptan (green triangles, 300 μg/kg, n = 8) treated rats. Values are means ± s.e.m. *Two-way ANOVA followed by Duncan's post hoc test, with time (P < 0.219) and treatment (P < 0.001) as factors.*

Boxplots (median of the sum of the values over the analyzed period) showing the antimigraine effect of PL37 (20 mg/kg, iv) and sumatriptan (300 μg/kg, iv) in rats receiving repeated ISDN injections. *Kruskal-Wallis one-way analysis of variance on Ranks with post-hoc Dunn's test. * P = 0.011, ** P = 0.002.*

Chronic migraine model – Oral administration – Prophylactic effect

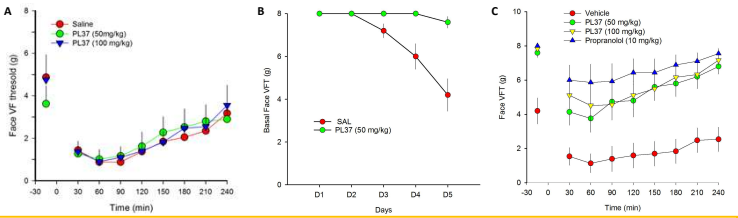


Figure 5: von Frey withdrawal thresholds of the face after repeated ISDN administration. (**A**) Single PL37 (50 mg/kg), PL37 (100 mg/kg) or Saline administration (**B**) Single PL37 (50 mg/kg) administration 5 min. before each ISDN administration from D1 to D5 shows that PL37 prevents chronic cephalic mechanical allodynia. (**C**) Time-dependent response on D5 after ISDN administration.

Single oral PL37 administration is inactive to reduce persistent cephalic mechanical allodynia in model induced by recurrent administration of ISDN but daily oral administration can act as prophylactic treatment (however, no dose-effect was observed between 50 and 100 mg/kg).

Chronic migraine model – Mechanism

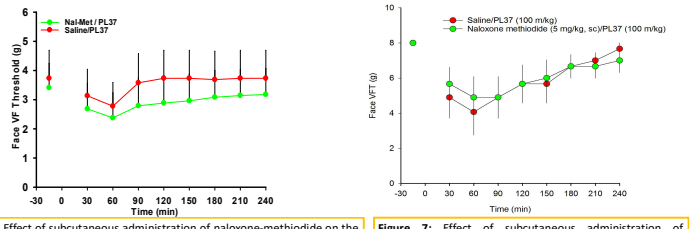


Figure 6: Effect of subcutaneous administration of naloxone-methiodide on the antiallodynic effect of intravenous PL37 in chronic model of migraine. Time courses of von Frey withdrawal thresholds (VFT) of the face in saline- (red circles, n = 8), naloxone methiodide (green circles, 5 mg/kg, n = 8) PL37 treated rats. Naloxone-methiodide or saline were administered subcutaneously (sc) 20 min before a new ISDN administration. Values are means ± s.e.m. Two-way ANOVA followed by Duncan's post hoc test, with *time* (P = 0.982) *and treatment (P < 0.001)* as factors.

Figure 7: Effect of subcutaneous administration of naloxone-methiodide on the antiallodynic effect of oral PL37 in chronic model of migraine on D5. Time courses of von Frey withdrawal thresholds (VFT) of the face in saline- (red circles), naloxone methiodide (green circles, 5 mg/kg, n = 8) PL37 treated rats. Naloxone-methiodide or saline were administered subcutaneously (sc) 20 min before a new ISDN administration. Values are means ± s.e.m.

Systemic naloxone-methiodide partially, but significantly reduced the antimigraine effect of the single iv PL37 (20 mg/kg) but did not reversed the antiallodynic effect of oral PL37 (100 mg/kg) in rats receiving repeated ISDN administration, demonstrating probably a central opioid receptor activation.

Conclusions

This study suggests that protecting enkephalins from degradation following administration of the DENKI® PL37 has pain alleviating effects in ISDN-induced migraine. Whereas single intravenous PL37 administration has an effect on chronic migraine model, oral administration has a prophylactic effect. Comparing the actions of oral and iv administration of PL37 suggests that both peripheral and central components are at play in regards to the results obtained using both the acute and the chronic migraine models. The results of this study further suggest that enkephalins play a key role in the modulation of trigeminal pain, probably through delta opioid receptors activation and indicate that DENKI® such as PL37 may represent an effective treatment of migraine as drug or prophylactic treatment.

References:

[1] Brennan KC, Pietrobon D, Neuron 2018; 97: 1004-1021; [2] Ashkenazi A, Sholtzow M, Shaw J, Burstein R, Young W, Cephalalgia 2007; 27: 111-117; [3] Burstein R, Yarnitsky D, Goor-Aryeth I, Ransil BJ, Bajwa ZH, Ann Neurol 2000; 47: 614-624; [4] Guy N, Marques A, Orliaquet T, Lanteri-Minet M, Dallel R, Clavelou P, Cephalalgia 2010; 30: 881-886; [5] Schwedt TJ, Krauss MJ, Frey K, Gereau MW, Cephalalgia 2001; 31: 6-12; [6] Boyer N, Dallel R, Artola A, Dallel R, Monconduit L, Pain 2014; 155: 1196-1205; [7] Boyer N, Signoret-Genest J, Artola A, Dallel R, Monconduit L, Pain 2017; 158: 2025-2034; [8] Jakubowski M, Levy D, Goor-Aryeth I, Collins B, Bajwa Z, Burstein R, Headache 2007; 27: 111-117; [9] Bigal ME, Lipton RB, Neurology 2008; 71: 848-855; [10] Bigal ME, Serrano D, Reed M, Lipton RB, Neurology 2008; 71: 559-566; [11] Roques BP, Fournie-Zaluski MC, Wurm M, Nature Rev Drug Discov 2012; 11: 292-310; [12] Menendez L, Hidalgo A, Meana A, Poras H, Fournie-Zaluski MC, Roques BP, Baamonde A, Eur J Pharmacol 2008; 596: 50– 55; [13] Poras H, Bonnard E, Dange E, Fournie-Zaluski MC, Roques BP, J Med Chem 2014; 57: 5748-5763; [14] Dallel R, Descheemaeker A, Luccarini P, Cephalalgia 2014; 57: 5748-576