LAT peptide motifs: a novel class of naturally derived peptide motifs and a target family of receptors that represents a unique, non-opioid, non-addictive mechanism for the treatment of neuropathic pain.

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INTRODUCTION

Pain management is of particular concern for the modern war fighter. Of the military personal that cannot be deployed for medical reasons, issues with pain management account for 70% of them; the equivalent of 10 million limited duty days each year. Neuropathic pain (NP) – pain associated with disease or damage to the somatosensory nervous system which can occur as a result of trauma or post-trauma surgery – has been reported to affect a quarter of soldiers wounded on the battlefield, representing a frequent and substantial cause of post-trauma disability. Available pharmacological therapies also exhibit modest efficacy along with their considerable side-effects, such as sedation, weight gain and fatigue, along with a significant risk of abuse and addiction posed by opioids: the prevalence of prescription opioid misuse among military personnel is reported to be twice as high as in the civilian population. Lateral Pharma has identified a novel class of naturally derived human peptide motifs (LAT peptides) and a target family of receptors that represents a unique mechanism for the treatment of neuropathic pain. The most advanced LAT peptide; LAT8881, has been well tolerated in animal toxicology studies and in Phase 1 and 2 clinical trials with 773 patients dosed to date. LAT8881 has low oral availability and a short half-life in blood after injection, with rapid distribution into all tissues except for the central nervous system.

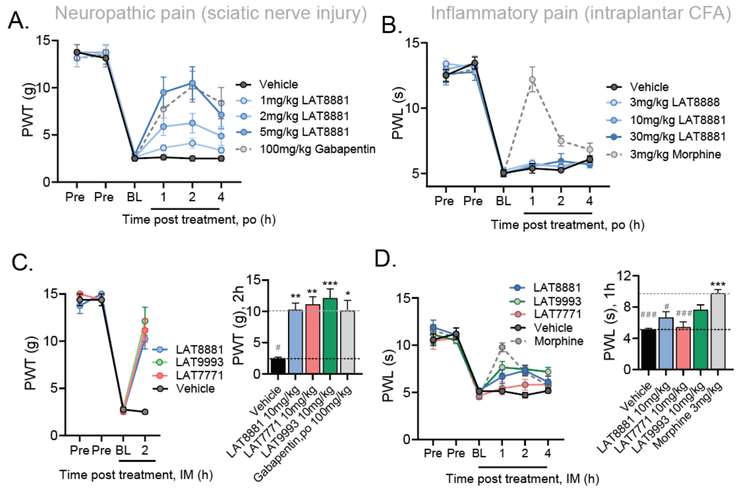
METHODS

Pain models: Rat chronic constriction injury (CCI) and Chung models were prepared by placing 4 loose ligations on the left sciatic nerve and by tightly ligating the L5 spinal nerve, respectively. To induce chemotherapy-induced neuropathic pain (CIPN), rats were treated with oxaliplatin for up to 4 weeks. Diabetic induced neuropathic pain was induced in rats by 1 or 2 injections of streptozotocin (50 mg/kg; i.p.). Reserpine-induced fibromyalgia models were developed by injecting reserpine (1 mg/kg; s.c.) for 3 consecutive days. Neuropathic pain status was confirmed by monitoring mechanical allodynia. Model rats were placed in individual plastic boxes on a metal mesh leaving the plantar surface exposed to von Frey hair testing. Baseline paw withdrawal threshold (PWT) was monitored and changes in PWT recorded before and up to 6 hours post-dosing. Lateral Pharma test compounds were administered intramuscularly (i.m.); IV or orally and Gabapentin was used as a positive control.

IHC: DRG were fixed in PFA and sections (20 µm) were prepared on a cryostat, incubated in phosphate buffer containing horse serum and Triton-X, before incubation in anti-LanCL1 and NeuN antibodies (Abcam). Sections were washed and incubated in fluorescent secondary antibodies, washed and imaged by confocal microscopy.

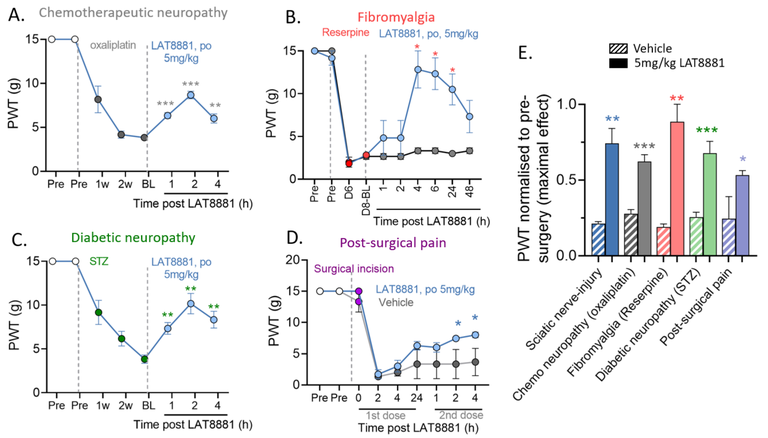
siRNA: siRNA for LanCL1 were purchased from Sigma and reconstituted based upon manufacturers instructions. A combination of sharp and blunt dissection was used to expose the lateral aspect of the fifth lumbar (L5) vertebrae and the intervertebral foramina. A small portion of laminar bone was removed exposing the DRG. 2 µl of LanCl1 or control scrambled siRNA were directly injected into the exposed L5 DRG and the wound closed. PWT was assessed before and 24, 48 and 72 hrs after siRNA injection. LAT8881 (10 mg/kg) was administered 2-3 days after siRNA injection into the DRG and PWT re-examined 1, 2 and 4 hours following dosing.

LAT compounds are analgesic in neuropathic, but not acute inflammatory pain models



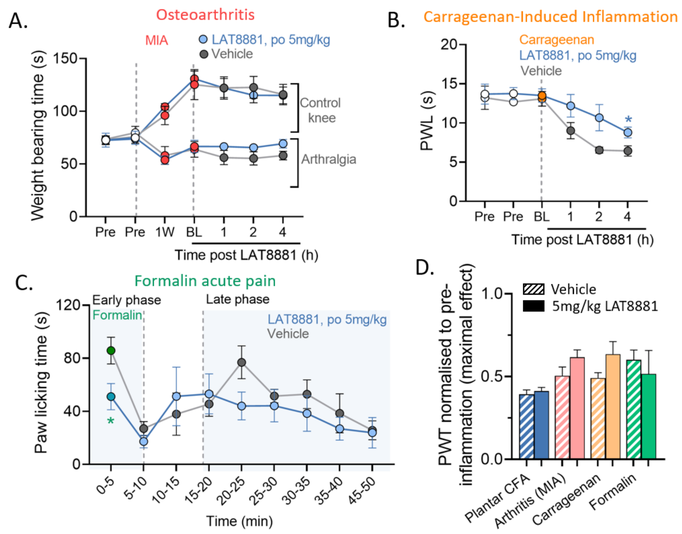
A. LAT8881, dosed orally (p.o.), reversed mechanical allodynia in Chung models of neuropathic pain in a dose-dependent manner. **B**. LAT8881, dosed via the intramuscular route (i.m.), was less effective in reversing hypersensitivity to heat in a model of acute inflammatory pain. **C**. Similar to LAT8881, LAT7771 and LAT9993 were also effective in reversing mechanical allodynia in Chung models of neuropathic pain. **D**. Also similar to LAT8881, LAT7771 and LAT9993 were less effective in acute inflammatory pain models.

LAT8881 reverses mechanical allodynia in multiple models of neuropathic pain models



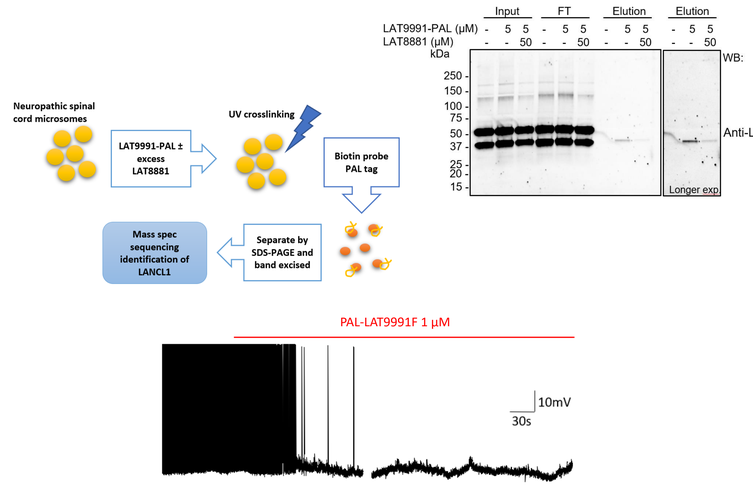
LAT8881, dosed orally, reversed mechanical allodynia in models of chemotherapy-induced neuropathic pain (oxaliplatin; (**A**), reserpine models of fibromyalgia (**B**), streptozotocin-induced diabetic neuropathy (**C**) and post-surgical pain (Brennan model; **D**). **E**). Summary comparison of the effectiveness of LAT8881 in different models of neuropathic pain with data normalised to baseline paw withdrawal thresholds (PWT; measured with von Frey hairs; force, g).

LAT8881 is less effective in models of inflammatory pain



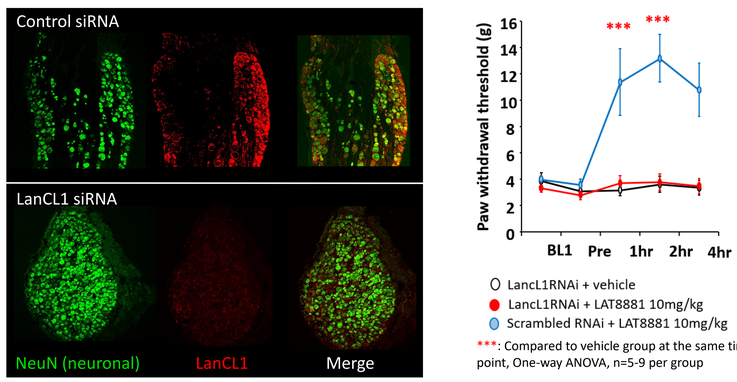
LAT8881, dosed orally, was less effective in models of inflammatory pain including: **A**, models of osteoarthritic pain (mono-iodoacetate (MIA), injected via the intra-articular route); **B**, formalin-induced acute inflammatory pain; **C**, Carrageenan-induced inflammatory pain. **D**. Summary comparison of the effectiveness of LAT8881 in different models of inflammatory pain with data normalised to pre-LAT8881 baseline behavioural readouts.

Target identification of LAT compounds: LanCL1



Top: Spinal cord microsomes were treated with LAT9991-PAL (5 µM) peptide in the absence or presence of LAT8881 (50 µM) followed by crosslinking between the PAL peptide with its interacting proteins by UV irradiation (365 nm for 20 min). After click chemistry reaction between the alkyne reporter tag on the PAL with an azido-biotin probe, biotin labelled proteins could be enriched with streptavidin beads. Input, FT and eluted proteins were separated on SDS-PAGE followed by WB with anti-LanCL1 and Streptavidin antibodies. **Bottom**: LAT9991F was validated electrophysiologically to behave in a similar manner to other LAT compounds, by inducing inhibition of spontaneous activity in spinal cord dorsal horn neurons *in vitro* in spinal cord slice/dorsal root preparations from Chung models of neuropathic pain.

LAT compound target validation: siRNA knockdown of LanCL1 abolishes effects of LAT8881



Top Left: Immunohistochemistry showing LanCL1 co-expression with DRG neurons (NeuN) from Chung models of neuropathic pain pre-treated with control, scrambled siRNA. **Below Left:** siRNA for LanCL1 suppressed LanCL1 expression in DRG. **Right**: Pre-treatment with siRNA for LanCL1 inhibited the effects of LAT8881 on mechanical allodynia in Chung models of neuropathic pain.

CONCLUSIONS

In conclusion, we identified a novel therapeutic target for neuropathic pain, LanCL1, and a unique class of naturally derived peptide motifs that act on this target to relieve mechanical allodynia associated with neuropathic pain. LanCL1 is selectively upregulated in multiple models of neuropathic pain and targeting LanCL1 with LAT peptides suppresses abnormal activity associated with the condition. These data suggest LanCL1 is a novel target for the treatment of neuropathic pain and offers a new opportunity to target this condition without the side-effects associated with current analgesics.