CLE-400: A Potent Analgesic Topical Gel in Acute and Chronic Pain Pig Models

Elanite Caspi, Johanna Schumann, Orna Goren, Elena Kagan, Clexio Biosciences, 17 Yegi'a Kapaim St., Petach Tikva, Israel

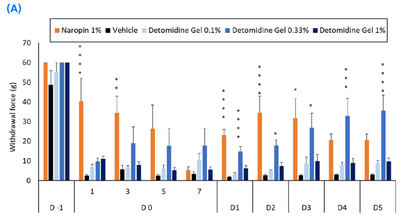
Background and Aims

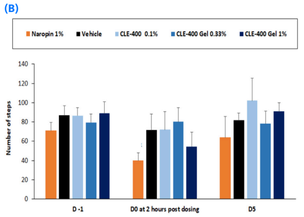
- Alpha 2 (α2) - adrenoceptor agonists have been in clinical use for decades, primarily in the treatment of hypertension. In recent years they have found wider application, particularly in the fields of anesthesia and pain management[1].
- Since α2-adrenoceptors were found to be present on nociceptors in the epidermis and dermis of the skin, topical use, which may limit systemic adverse events without loss of analgesic effect, have gained interest[2].
- CLE-400 is a new investigational drug that has not been approved for commercial distribution. CLE-400 is a novel topical formulation of detomidine, a potent α2-adrenoceptors agonist used for many years in veterinary medicine as a systemic medication for sedation and analgesia. CLE-400 was developed with a proprietary topical formulation to enable maximal skin penetration while limiting systemic exposure.
- The aim of these preclinical studies was to examine the analgesic effect of CLE-400 for the management of acute post-operative pain and chronic neuropathic pain in pig models. Furthermore, studies were set to determine if there are additional protein targets regulated by detomidine, whether they are expressed in skin and thus, potentially associated with local analgesia.
- All experimental procedures were approved by an Institutional Animal Care and Use Committee in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Efforts were taken to minimize pain and distress of experimental animals.

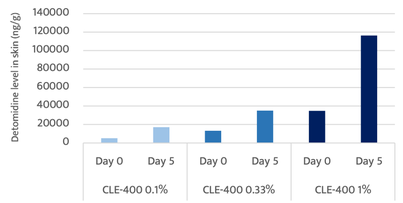
Methods

- The validated post-operative pain pig model was selected as the preclinical model for acute pain[3]. The pig was chosen for this study due to the notable anatomic, physiologic and neurologic resemblance between pig skin to human skin. Briefly, pigs underwent full-skin incision of 6–7 cm through the skin and fascia, keeping the muscle intact. Starting from fifteen minutes following surgery, CLE-400 at 0.1%, 0.33% and 1% strengths or placebo were administered, twice daily, at a volume of 3 µl/cm^2 over an area of ~50 cm^2 for 6 consecutive days. Subcutaneous injection of Naropin was used as a positive control. Mechanical sensitivity was assessed using the von Frey methodology together with General Behavior Scoring (GBS) to assess spontaneous pain and open field test to assess motor activity. These tests were performed 1 day pre-surgery (baseline), 1h, 3h, 5h and 7h post-dose on Day 0 and then once daily for an additional 4 days (1 hour post-dose). Additionally, on Days 0 and 5 of study, blood samples and skin biopsies collected from the administration site, were taken in order to determine CLE-400 (detomidine) levels in skin and plasma.
- The validated Peripheral Neuritis Trauma (PNT) model developed in domestic pigs[4] was chosen to evaluate CLE-400 effect on chronic neuropathic pain. Briefly, two weeks following PNT induction by partial sciatic nerve ligation, animals exhibiting mechanical and tactile allodynia were topically administered with CLE-400 at 0.1%, 0.33% and 1% strengths or placebo at 50 µL/cm^2 over a 80 cm^2 area, twice daily for 14 consecutive days on the dorsal part of the low foot - the area that is innervated by the injured sciatic nerve. Intravenous injection of Gabapentin served as a positive control. Mechanical sensitivity was assessed using the von Frey methodology and tactile allodynia was evaluated using the feather test at various time points post-treatment. Additional assessments and scoring methods were conducted to assess spontaneous pain-like animal behaviors including GBS.
- A panel of *in vitro* radioligand binding and functional cell based assays was performed to assess the pharmacological activity of detomidine on additional targets. Briefly, the activity of detomidine was evaluated in radioligand binding assays against 172 molecular targets (G-protein coupled receptors, ion channels, transporters, enzymes) at a single concentration (10 µM). Targets showing significant binding to detomidine were further investigated in functional assays at several concentrations for IC_{50} or EC_{50} determination.
- The presence of primary and secondary detomidine targets was further assessed in pig skin biopsies from the PNT study by immunohistochemistry analysis.

Figure 1 Analgesic efficacy in acute post-operative (POP) pig model





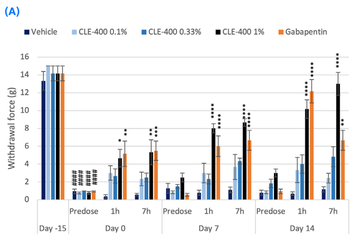


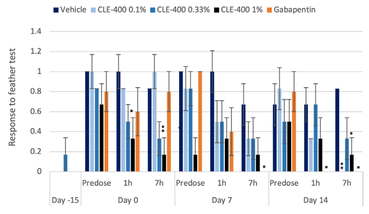
(D) Skin/plasma ratio

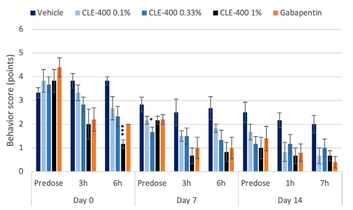
	Day 0	Day 5
CLE-400 0.1%	353	771
CLE-400 0.33%	301	384
CLE-400 1%	392	1086

(A) Topical application of 0.33% CLE400, administered twice daily, for 6 days, following skin incision, exhibited a substantial analgesic effect by significantly increasing mechanical withdrawal force (von Frey) and demonstrating a cumulative analgesic effect over time as compared to placebo. Mean group response to von Frey (g) stimuli. *P<0.05; **P<0.01; ***p<0.001; ****p<0.0001 vs. placebo using one-way ANOVA followed by Tukey's test. **(B)** CLE-400 gel had no significant effect on locomotor activity, indicative of a lack of systemic toxicity, such as sedation. Mean group number of steps (meters) (open field) **(C)** Detomidine concentration in skin increased throughout the study and was dose proportional. **(D)** Analysis of skin and plasma levels of detomidine revealed high concentrations of detomidine in the skin compared to plasma, suggesting that the skin may act as a depot, slowly releasing detomidine into the circulatory system. Treatment with Naropin (orange column) (control), significantly increased withdrawal force (A) and had no significant effect on locomotor activity (B).

Figure 2 Analgesic efficacy in PNT chronic neuropathic pain pig model

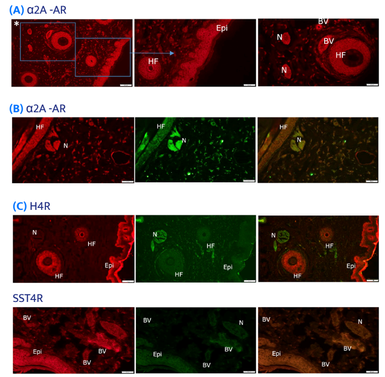






(A) Topical treatment of CLE-400 1%, twice daily, for 14 days resulted in significant cumulative analgesic effect, as measured by increasing withdrawal force in a von Frey test. #### p<0.0001 using paired Student's T-test vs. baseline. *p<0.05; **p<0.01; ***p<0.001 ****p<0.0001 vs. Vehicle using one-way ANOVA followed by Tukey's test **(B)** CLE-400 1% reduced the number of responders to feather test at all study time points, indicative of reduced mechanical allodynia in response to light touch stimulation *p<0.05; **p<0.01; vs. Vehicle using Fisher Exact Test. **(C)** Treatment with CLE-400 resulted in a dose-dependent decrease in the behavior score (animals less distressed) starting several hours post treatment, with CLE-400 1% being significantly lower as compared to placebo-treated animals (black column) as soon as Day 0 and up to Day 14. *p<0.05; ***p<0.001 vs. Vehicle using Kruskal-Wallis nonparametric test. Treatment with Gabapentin (orange column) (control) significantly increased withdrawal force (A), reduced response to feather test (B) and reduced the behavior score (C).

Figure 3 Detomidine targets α2A-adrenoceptor, histamine 4 receptor and somatostatin 4 receptor are expressed in pig skin



(D)

Receptor	EC_{50} (agonist)
$α2_A$ adrenergic (h)	23.5 nM
h_4 (h)	4.7 µM
sst_4 (h)	7.3 µM

Identification of α2A-adrenoceptor (α2A-AR), histamine 4 receptor (H4R) and somatostatin 4 receptor (SST4R) expression in pig skin by immunohistochemistry staining. **(A)** α2A-AR (red) is expressed in epidermis (full thickness) (epi), hair follicles (HF), blood vessels (BV) and nerves (N). **(B)** α2A-AR (red) co-localizes with PGP9.5 (pan-neuronal marker) (green). **(C)** H4R (red) is expressed in HF and lower cells of epidermis. SST4R expression (red) is seen in epidermis, BV, HF and nerves (co-localizes with PGP9.5 (green)). All images were taken using x40 objective, total magnification x400, scale bar 50µm. Image (*) was taken using x20 objective, total magnification x200, scale bar 100µm. **(D)** EC_{50} values of detomidine targets.

Conclusion

CLE-400 appears promising as a non-systemic, topical formulation for treating acute and chronic pain. Topical administration of CLE-400 demonstrated a rapid and cumulative analgesic effect in acute pain and chronic neuropathic pain pig models. CLE-400 gel formulation maintains high dermal concentrations over an extended period of time (depot effect in the skin) while maintaining limited systemic exposure. Lastly, CLE-400 may activate, in addition to α2 adrenoceptors, the histamine 4 receptors and somatostatin 4 receptors which are all expressed in skin and thus, potentially associated with the achieved local analgesia.

References

1. Giovannitti JA, Thoms SM, Crawford JJ. Alpha-2 adrenergic receptor agonists: a review of current clinical applications. Anesth Prog . Spring 2015; 62(1):31-9. doi: 10.2344/0003-3006-62.1.31.
2. Riedl MS, Schnell SA, Overland AC, Chabot-Doré AJ , Taylor AM, Ribeiro-Da-Silva A, Elde RP, Wilcox GL, Stone LS. Coexpression of α2A-adrenergic and δ-opioid receptors in substance P-containing terminals in rat dorsal horn. J. Comp. Neurol. 2009; 513(4):385–398. doi:10.1002/cne.21982.
3. Castel D, Willentz E, Doron O, Brenner O, Meilin S. Characterization of a porcine model of post-operative pain. Eur J Pain 2014; 8(4):496-505. doi:10.1002/j.1532-2149.2013.00399.x.
4. Castel D, Sabbag I, Brenner O, Meilin S. Peripheral Neuritis Trauma in Pigs: A Neuropathic Pain Model. J. Pain 2016;17(1):36-49. doi.org:10.1016/j.jpain.2015.09.011.