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Pharmacological Effects of ACD137, a Small Molecule Negative Allosteric Modulator of TrkA

Forsell, P., Nordvall, G., Backlund, M., Lidell, V., Rasti, A., Parrado-Fernandez, C., Sandin, J. and **Segerdahl, M.**, AlzeCure Pharma AB, Hälsovägen 7, Huddinge, Sweden

Aim
The aim of the present study was to evaluate ACD137, a TrkA-NAM compound, for its pharmacological effects in various pain models.

Background
Negative allosteric modulators (NAMs) targeting Tropomyosin receptor kinase A (TrkA), a pivotal receptor in neurotrophin and pain signaling, represent a promising avenue in therapeutic intervention. By binding to a site distinct from the ATP-binding site on TrkA [1, 2], NAMs offer a unique approach to attenuate TrkA signaling without directly interfering with the levels of NGF.

Methods
In vitro pharmacological studies were performed using the intracellular domain of TrkA or U2OS cells overexpressing TrkA. In vivo models including chemotherapy-induced peripheral neuropathy (CIPN) and the Brennan model (incisional pain) were performed using male Sprague Dawley rats. CIPN was induced by Paclitaxel administration by i.p. injection on day 0, 2, 4, and 6. Incisional pain was studied according to the Brennan model by performing a 1 cm incision, involving a longitudinal cut of the plantaris muscle, of the right hind paw in anesthetized rats. Osteoarthritis (OA) pain was studied by local injection of mono-iodo acetate (MIA) in the left knee joint to induced OA-like pathology. Mechanical allodynia was assessed by withdrawal threshold using von Frey filaments or Dynamic Plantar Aesthesiometer.

Ethical Permissions
The in vivo studies were performed at Aragen Life Science or at SAI Life Sciences, India. The protocols were approved by the Institutional Animal Ethics Committee (IAEC) (B106-56-23-IPH, B048-75-23-IPH, FB-24-02)

Results
ACD137, a potent, selective, and orally bioavailable TrkA-NAM with a Kd of 0.011 nM and a mean IC_{50} value of 0.8 nM on TrkA and >20,000 fold selectivity over TrkB in a cell-based assay, was synthesized in our laboratory during a lead optimization program. The protein crystal structure with an in-house TrkA-NAM compound co-crystalized to the intracellular domain of TrkA was solved by x-ray crystallography (fig 2). The binding site was found to be overlapping with that reported by Pfizer (compound #23; pdb 6D20) [2]. Analgesic efficacy was tested in several models including paclitaxel-induced peripheral neuropathy, post-operative/incisional pain and OA-like pain. Oral treatment with ACD137 resulted in a significant increase in paw withdrawal threshold when compared to vehicle treated control animals in all three models, thus demonstrating a broad analgesic effect covering both neuropathic and nociceptive pain.

Discussion
TrkA-NAM's have shown analgesic effects in both neuropathic and nociceptive models, potentially without the side effects and dependency issues observed for opioids. Enhanced pain relief is attributed to the inhibition of NGF/TrkA signaling, attenuating peripheral sensitization and neuroinflammation. Identification of selective and potent TrkA-NAM's could potentially avoid some of the side effects observed for anti-NGF antibodies due to a more selective mechanism of action, while retaining the analgesic efficacy. Molecules that selectively inhibit TrkA will avoid any side effects related to inhibition of proNGF- or NGF-mediated p75NTR-signaling that anti-NGF antibodies potentially could suffer from. We have previously shown that ACD137 has analgesic and anti-inflammatory effects. The present study further clarifies the mechanism of action and supports the analgesic effect of ACD137 in relevant preclinical models for both neuropathic and nociceptive pain. Overall, TrkA-NAMs demonstrate potential as a novel therapeutic approach for alleviating different types of pain, including OA-pain, highlighting their role in advancing pain management strategies.

References
1. Furuya, N., et al., The juxta membrane region of TrkA kinase is critical for inhibitor selectivity. Bioorg Med Chem Lett, 2017. 27(5): p. 1233-1236.
2. Bagal, S.K., et al., Discovery of Allosteric, Potent, Subtype Selective, and Peripherally Restricted TrkA Kinase Inhibitors. J Med Chem, 2019. 62(1): p. 247-265.

Figure 1. IC50 curves of ACD137 on TrkA or TrkB

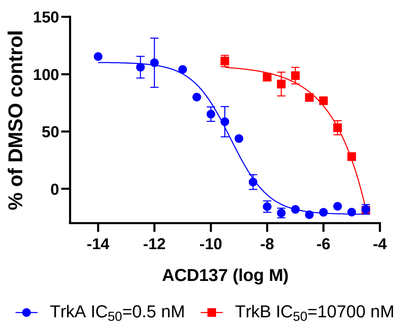

Figure 1.
U2OS-TrkA/SHC1-p75 or U2OS-TrkB/SHC1-p75 cells treated with NGF or BDNF (10 ng/ml) and ACD137 at the indicated concentrations. Chemiluminescence was detected using the PathHunter® detection reagent provided in the kit. Results are the mean +/- SEM (n=2).

Figure 2. TrkA-NAM binding site

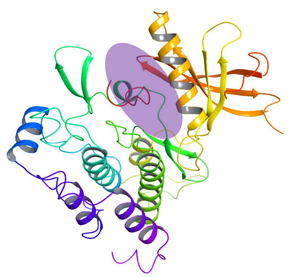

Figure 2.
X-ray structure of the intracellular domain of TrkA
The shaded area represents an internal TrkA-NAM molecule co-crystalized to TrkA. The binding site is overlapping with previously reported binding site for TrkA-NAM molecules [2].

Figure 3. Effects of ACD137 on neuropathic pain

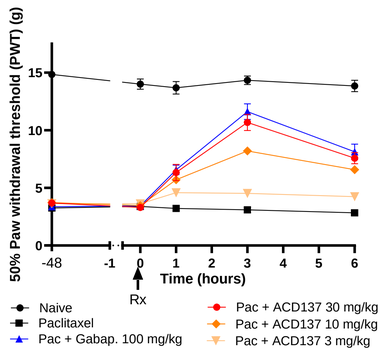

Figure 3.
Intraperitoneal injection of Paclitaxel was performed on day 0 (after baseline recording). Paw withdrawal threshold was measured on Day13 after induction (pre-treatment baseline). On Day 15, rats were treated with vehicle (black), ACD137 (red, orange and light orange) or gabapentin (blue), and mechanical allodynia was assessed at 0, 60 min, 180 min and 360 min after treatment. Results are the mean +/- SEM (n=10 animals in each group).

Figure 4. Effects of ACD137 on incisional pain

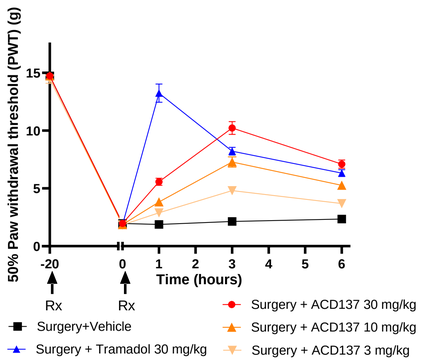

Figure 4.
A surgical paw incision, following the method outlined by Brennan, was performed on each animal. Paw withdrawal threshold was assessed 30 minutes before the surgical procedure and then again 20 hours following the surgery. Animals were treated with vehicle (black), ACD137 (red, orange and light orange) or tramadol (blue), and mechanical allodynia was assessed at 0, 60 min, 180 min and 360 min after treatment. Results are the mean +/- SEM (n=12 animals in each group).

Figure 5. Effects of ACD137 on OA-like pain

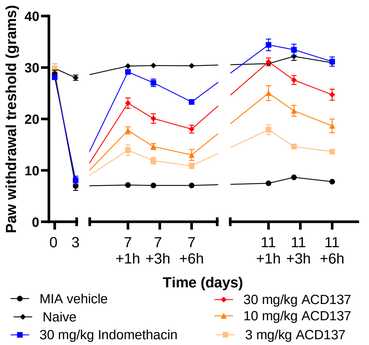

Figure 5.
On day 0, osteoarthritis was induced by intraarticular injection of 2 mg/mL MIA at a dose volume of 50µL to left hind limb. On day 3, mechanical allodynia was measured and the animals showing 50% reduction in the basal paw withdrawal threshold were randomized into groups. From day 3 to day 11, animals were administered ACD137 once daily and mechanical allodynia was assessed by using Dynamic Plantar Aesthesiometer (Ugo Basile) at maximum time force 50 g and ramping time 20sec.
Results are the mean +/- SEM (n=8 animals in each group).

Conclusion
The NGF/TrkA pathway is a well validated pathway for new analgesics without the side effects and dependency issues observed for opioids. Identification of selective TrkA-NAMs could potentially avoid some of the side effects observed for anti-NGF antibodies or non-selective Trk-inhibitors, while retaining the analgesic efficacy. We have identified potent and selective TrkA-NAMs demonstrating analgesic and anti-inflammatory effects in vivo in both models of neuropathic and nociceptive pain.

Contact
AlzeCure Pharma AB, Hälsovägen 7, 141 57 Huddinge, Sweden | Martin.jonsson@alzecurepharma.com | www.alzecurepharma.com