Effects on neuroprotection and neuroplasticity by the clinical compound ACD856, a novel positive modulator of Trk-receptors from the NeuroRestore® platform

Johan Sandin, Sanja Juric, Cristina Parrado-Fernández, Nather Madjid, Gunnar Nordvall, Maria Backlund, Märta Dahlström, and Pontus Forsell,
AlzeCure Pharma AB, Hälsovägen 7, Huddinge, Sweden

Objectives

Given that the neurotrophins are known to exert neuroprotective effects and to increase neuronal plasticity, the major objective of this study was to assess the effects of the clinical stage compound ACD856, a positive allosteric modulator of Trk-receptors, on neuronal plasticity and neuroprotection.

Background

Positive allosteric modulators of Trk-receptors is a promising mechanism for increasing the efficacy of neurotrophin signaling thereby lending neurotrophic support and improved synaptic function to neurons with compromised function.

Methods

In vitro effects of ACD856 were investigated in embryonal primary cortical or hippocampal neurons by measuring metabolic activity, phosphorylation of ERK1/2 and synaptic markers. Effects on synaptic markers were also studied in PC12 cells. Effects on neuronal plasticity were further investigated in a fear-conditioning cognition model and a model of depression in vivo.

Results

Energy-deprivation can be used to study effects on improved mitochondrial function. Thus, cortical neurons were deprived of glucose and pyruvate as energy sources relying only on the tricyclic acid cycle and oxidative phosphorylation as means for ATP production. In this setting, ACD856 demonstrated increased ATP-levels (data not shown) and was protective against energy-deprived induced neurotoxicity (fig. 1). In order to investigate how cortical neurons respond to different neurotrophic factors, we investigated the phosphorylation of ERK1/2 after the addition of different factors. The neurotrophins BDNF and NT-3 had a substantial effect on phospho-ERK1/2 levels in cortical neurons when compared to other neurotrophic factors (fig. 2).
Interestingly, ACD856 demonstrated a positive effect on the levels of phosphorylated ERK1/2 in neurons, suggesting that one of the effects of the compound is to modulate phospho-ERK1/2 levels (fig. 3). Furthermore, the synaptic protein SNAP25 was increased in PC12 cells after treatment with ACD856 (fig 4), suggesting that ACD856 can improve synapse function or neuronal plasticity. Repeated administration of ACD856 suggest a long-term effect on neuronal plasticity, as judged by enhanced cognitive abilities at lower doses (fig. 5), and a sustained anti-depressant effect 3 days after the last dose (fig. 6).

Figure 1. Neuroprotective effects of ACD856

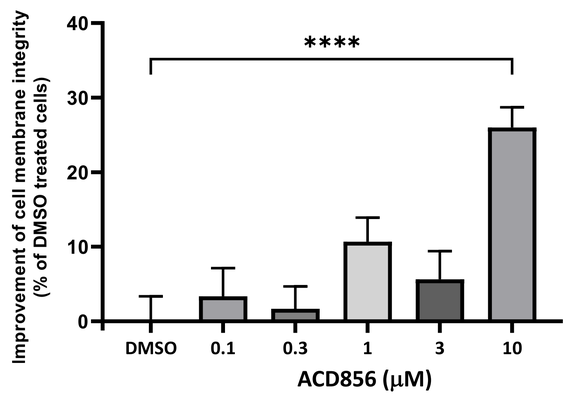

Figure 1. Primary cortical neurons were used to address to effect of ACD856 on cell membrane integrity 4 hours after removal of glucose and pyruvate in cell media. The fluorogenic peptide substrate (bis-AAF-R110) is not cell permeable in live cells. The amount of fluorescence can thus be correlated to the cellular membrane integrity. Data shown is normalized to DMSO-treated cells and the results are the mean +/- SEM. n=4. ****p < 0.001

Figure 2. Ligand-induced ERK1/2 and Trk-phosphorylation

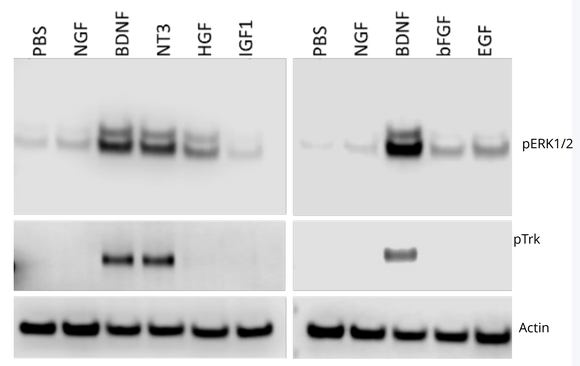

Figure 2. Western blot analysis of homogenates of cortical neurons stimulated with 10 ng/mL of NGF, BDNF, NT3, HGF, IGF1, bFGF or EGF. Upper panels show phosphorylated ERK1/2, middle panel shows phosphorylated Trk-receptors (Y490) and lower panels is a loading control.

Figure 3. ACD856 increases pERK1/2 in cortical neurons

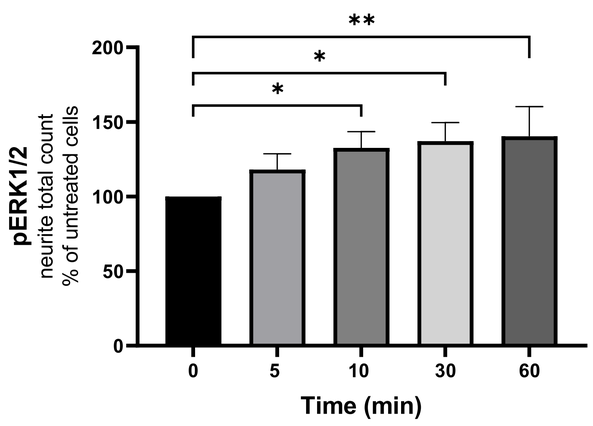

Figure 3. Primary cortical neurons were stimulated with 1 μM ACD856. Thereafter, cells were fixed with paraformaldehyde. Immunocytochemistry was performed using a rabbit anti-phospho ERK1/2 antibody followed by an anti-rabbit Alexa647-labelled antibody. Images were aquired using an ArrayScan automated HCS system. Data shown is the mean +/- S.D. n=3. *p < 0.05, **p < 0.01

Figure 4. ACD856 induces SNAP25 expression in PC12 cells

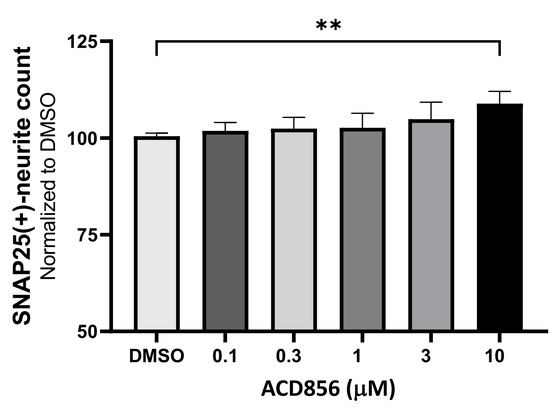

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Figure 5. Effects of repeated administration of ACD856

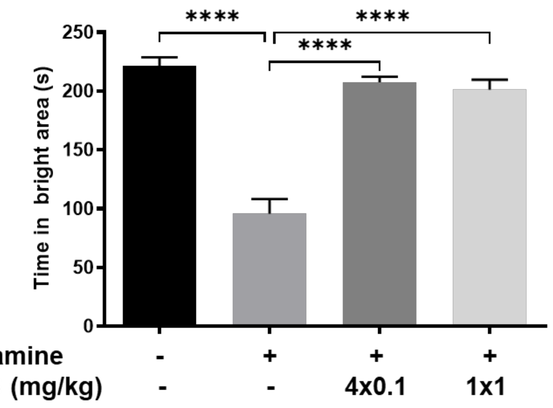

Figure 5. C57Bl/6J mice were subcutaneously administered ACD856 repeatedly for 4 days (0.1 mg/kg/day) or with a single administration (1 mg/kg) followed by scopolamine (0.3 mg/kg) 30 minutes prior to training in a fear-conditioning-based model. Data shown in each group is the mean +/- SEM, n=8. ****p < 0.0001

Figure 6. Sustained antidepressive-like effect of ACD856

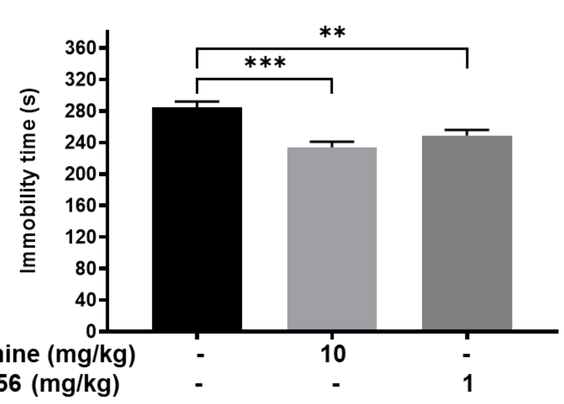

Figure 6. Animals were treated with ACD856 for 4 days or with ketamine for one day by subcutaneous injections. Antidepressive-like effects were assessed in the forced swim test in C57Bl/6J mice three days after the last administration. Data shown in each group are the mean +/- SEM, n=8. **p < 0.005, ***p < 0.001

Conclusion

The results indicate that ACD856, a compound in clinical development and part of AlzeCure's NeuroRestore platform, can act in a neuroprotective manner, modulate phospho-ERK1/2 levels and improve neuronal plasticity or in other ways increase network connectivity. The fact that ACD856 shows both symptomatic and potential disease-modifying effects is of high importance for the future treatment of patients with Alzheimer's disease and other neurodegenerative disorders.

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Hälsovägen 7, 141 57, Huddinge, Sweden

martin.jonsson@alzecurepharma.com
www.alzecurepharma.com