LP095

NeuroRestore ACD856, a Trk-PAM in clinical development for Alzheimer's disease shows neuroprotective and neurorestorative effects

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Objectives

The objective of these studies was to assess whether ACD856, a positive modulator of Trk-receptors, displays any effect on neuroprotection or neuronal plasticity that would support potential disease-modifying effects of this novel molecule.

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Background

Neurotrophins are a family of proteins that play a crucial role in the development, maintenance, and survival of neurons in the nervous system. One of the most well-known neurotrophins is brain-derived neurotrophic factor (BDNF), and others include nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). They bind and mediate their effects through the tropomyosin-receptor kinase (Trk) receptors (TrkA, TrkB and TrkC). A large body of scientific data suggests that neurotrophins could play a significant role in Alzheimer's (AD) and other neurodegenerative diseases, mediating effects on neuronal survival and plasticity, cognitive function, neuroregeneration and neuroprotection.

AlzeCure Pharma has developed first-in-class novel positive allosteric modulators of Trk-receptors and the lead candidate ACD856 has recently successfully completed phase I clinical trials. This compound has previously shown potent cognitive-enhancing properties in several preclinical animal models as well as antidepressant-like effects.

Figure 1. ACD856 enhances NGF-induced neurite outgrowth in PC12 cells

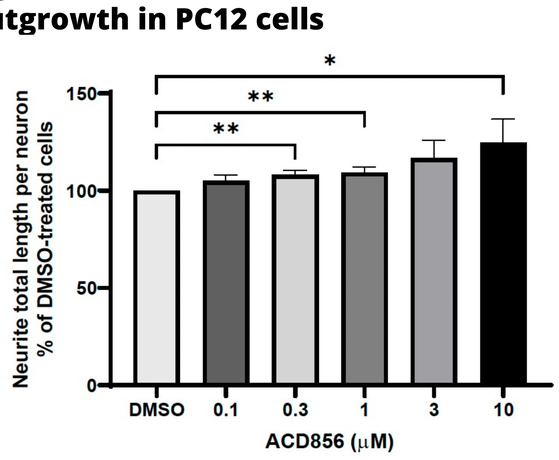

Figure 1. PC12 cells were treated with 3 ng NGF/mL for 5 days in the absence or presence of increasing concentrations of ACD856. Anti-tubulin antibodies were used to visualize neurite processes. Data shown are the mean of average values from each experiment +/− SD (n = 5 independent experiments), p-value < 0.05, ** p-value < 0.01 compared to control group.*

Methods

PC12 cells were incubated with DMSO or increasing amounts of ACD856 in the presence of 3 ng NGF/mL for 5 days and neurite outgrowth, as measured by neurite total length, was studied. SNAP25 levels were analyzed in NGF-treated PC12 cells in the presence of increasing concentrations of ACD856. Neuroprotective effects were studied using primary cortical cells exposed to 10 μM Aβ1-42 for 96 h with or without ACD856, and the levels of SNAP-25 in neurites were determined by immunocytochemistry. BDNF levels in vitro was assessed using primary cortical neurons incubated with increasing concentrations of ACD856, and the levels of BDNF were thereafter determined. For the in vivo BNDF studies, twenty-one months old C57Bl/6J mice were dosed with 5 mg/kg ACD856 once daily for 4 weeks by s.c. injection. The left hemisphere of each brain was homogenized, and the BDNF levels were determined by ELISA.

Figure 2. ACD856 increases the number of SNAP25-positive neurites in PC12 cells

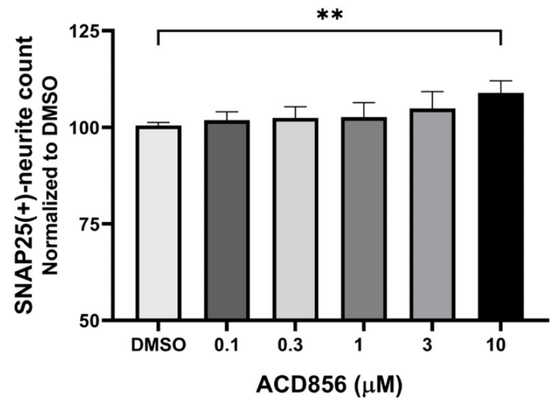

*Figure 2. PC12 cells were treated with 3 ng NGF/mL for 5 days in the absence or presence of increasing concentrations of ACD856. Anti-SNAP25 antibodies were used for detection. Data shown are the mean of average values from individual experiments +/− SD (n = 3), ** p-value < 0.01.*

Figure 3. ACD856 protects cortical neurons from Aβ42-induced toxicity

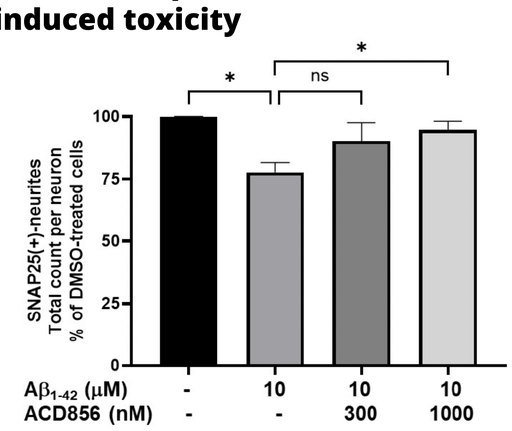

Figure 3. Primary neurons were treated at DIV 13 with vehicle (DMSO), 10 μM Aβ1-42, or different concentrations of ACD856 + 10 μM Aβ1-42 for 96 h in NB-medium supplemented with B27. The levels of SNAP25 were visualized and quantified by immunocytochemistry. Data shown are the means +/− SD of the average results from four independent experiments where each individual condition was performed using six different wells. The data are from four different preparations of cortical neurons. p-value < 0.05.*

Results

In the functional in vitro studies, ACD856 enhanced NGF-induced neurite outgrowth, both as measured by neurite total length per neuron (Fig. 1) and by neurite total length per well. Furthermore, ACD856 also increased the levels of the pre-synaptic protein SNAP-25 in neurites (Fig. 2). A closer analysis revealed that the SNAP25-protein was mainly localized to the cell body, neurites, and especially in buddings of neurites or at nerve endings.

In the neuroprotection assay, ACD856 was able to significantly protect the cortical neurons from Aβ42-induced toxicity (Fig. 3). Furthermore, ACD856 increased the levels of BDNF in both isolated nerve cells (Fig. 4A) and in the brain of aged animals (Fig. 4B), which have a natural reduction in the levels of BDNF. Combined, these studies suggest that ACD856 has neuroprotective and neurorestorative properties.

Figure 4A. ACD856 increases BDNF levels in PCN

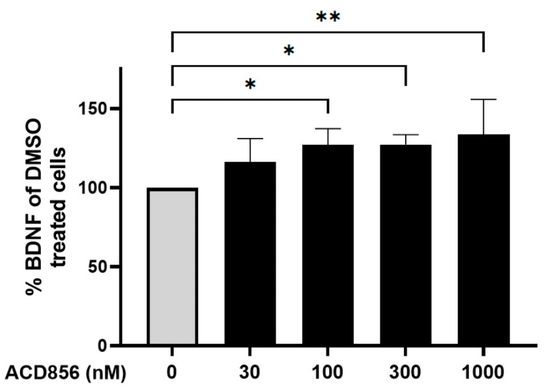

*Figure 4A. Cortical neurons were incubated with ACD856 in NB media for 6 and the BDNF levels were determined by ELISA. Data shown are the mean +/− SD from four independent experiments where each experiment was performed using six technical replicates. Each sample was analyzed in duplicates in the ELISA assay. * p < 0.05, ** p < 0.01 vs. DMSO-control values.*

Figure 4B. ACD856 increases BDNF levels in aged mice

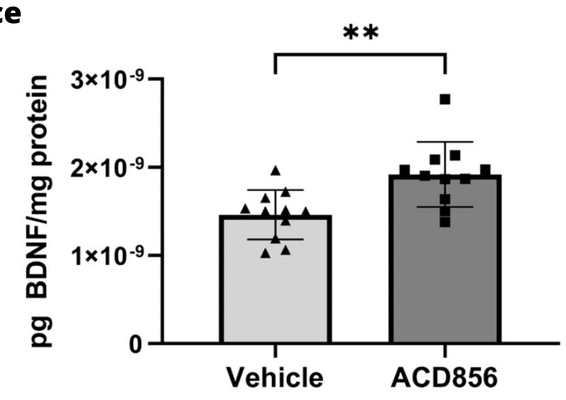

*Figure 4B. Twenty-one months old mice were dosed with 5 mg/kg ACD856 once daily for 4 weeks by s.c. injection. The left hemisphere of each brain was homogenized, and the BDNF levels were determined by ELISA. Data shown are the mean +/− SD, n = 11–12 animals. All samples were analyzed in duplicates in the ELISA assay, and BDNF levels were normalized to protein content in each sample. Individual data for each animal is the means of duplicate determinations. ** p < 0.01 vs. vehicle-treated animals. Light grey bar and black triangles indicates vehicle treated animals whereas as dark grey bar and black squares indicated ACD856 treated animals..*

Conclusion

We established that the Trk-PAM NeuroRestore ACD856 had neurotrophic effects, stimulating neurite outgrowth and increasing the levels of the presynaptic protein SNAP25 in PC12 cells. Additionally, it displayed neuroprotective effects, significantly decreasing Aβ1-42-induced toxicity. ACD856 was also able to increase levels of BDNF in vitro and in vivo suggesting effects that are long lived due to the function and role of BDNF on surrounding tissues. These data support potential disease-modifying effects of ACD856, which combined with cognitive-enhancing properties, would provide a step-change in future therapy management for Alzheimer's patients and potentially also in other neurodegenerative diseases, to protect against or delay disease progression.

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