LP024

Preclinical characterization of ACD856, a cognitive enhancer in clinical development for the treatment of cognitive dysfunction in Alzheimer's disease, demonstrates increased plasticity, neuroprotection and a possible disease modifying effect.

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Objectives

The objectives of these studies were to assess whether ACD856 exerts any effect on neuronal plasticity and to investigate possible disease-modification effects of the compound in vitro and in vivo.

Background

Neurotrophins are a class of growth factors that regulate neuronal function, survival, differentiation and plasticity. The neurotrophins, including nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and neurotrophin (NT) 3 and NT-4/5, bind and mediate their effects through the tropomyosin-related kinase (Trk) family of receptor tyrosine kinases (TrkA, TrkB and TrkC).

AlzeCure Pharma has developed a novel positive modulator of Trk-receptors, ACD856, which is in clinical development as a cognitive enhancer for Alzheimer's disease. Given the neuroprotective and neuroregenerative properties of neurotrophins, ACD856 may also have potential disease-modifying effects in AD.

Methods

In vitro effects of ACD856 were evaluated in cell lines or in primary cortical neurons. NGF-induced neurite outgrowth was studied by immunocytochemistry. Expression levels of pTrKB and BDNF were evaluated by ELISA. Effects on ATP levels were investigated in primary cortical neurons (PCN) using an energy deprivation-induced neurotoxicity assay. In vivo cognitive effects were studied using the passive avoidance model and the antidepressant-like effects were studied in mice using the forced swim test.

Fig 3. ACD856 increases neurite length (A) and SNAP25-positive neurites (B)

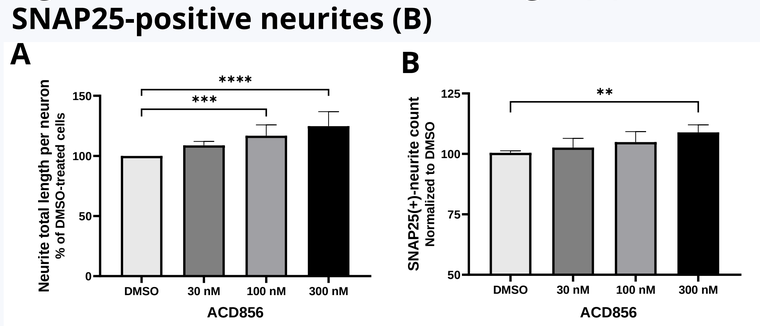

Figure 3. PC12 cells were incubated with ACD856 and 3 ng/ml of NGF 4-6 days. Fixed cells were immunostained with anti-Tubulin III (A) or SNAP25 (B) antibodies. Data shown are the means from five (A) or three (B) independent experiments +/- SD.

Results

ACD856 acts as a potent positive modulator of human Trk-receptors and enhances both NGF/TrkA signalling (fig. 1) and phosphorylation of TrkB in SHSY5Y-TrkB cells (fig 2). Functional in vitro effects of ACD856 in vitro includes increased neurite outgrowth (fig 3A), enhanced expression of SNAP25 (fig 3B) and enhanced levels of BDNF in mouse PCN (fig. 4), suggesting neurorestorative properties. Furthermore, ACD856 exhibits neuroprotective effects in a model of energy deprived-neurotoxicity (fig 5). ACD856 demonstrated sustained antidepressant-like effects in the forced swim test, even up to three days after the last administration (fig 6). ACD856 could also reverse scopolamine-induced memory impairment, at a significantly lower dose after repeated dosing as compared to a single administration (fig 7). The minimal effective dose for a single administration was determined to be 0.3 mg/kg. Furthermore, repeated administration was without any effects on motor function or anxiety.

Fig 1. ACD856 increases TrkA signaling in U2OS-cells

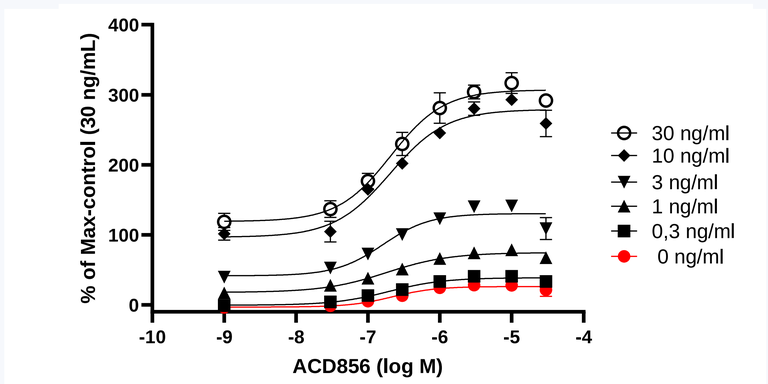

Figure 1. U2OS-TrkA cells were incubated with ACD856 and increasing concentrations of NGF, n=4 +/- SEM.

Fig 4. ACD856 increases the BDNF levels in PCN's

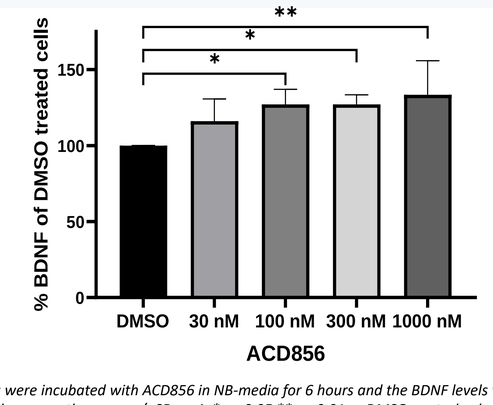

*Figure 4. PCN's were incubated with ACD856 in NB-media for 6 hours and the BDNF levels were determined by ELISAData shown are the mean +/- SD, n=4. *p < 0.05 **p < 0.01 vs DMSO-control values.*

Fig 6. Sustained antidepressive-like effects of ACD856

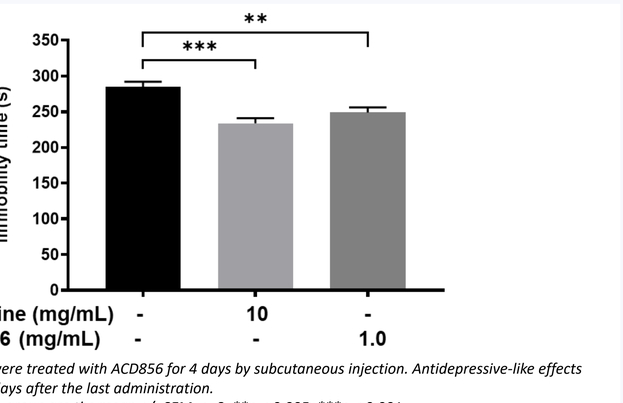

Figure 6. Animals were treated with ACD856 for 4 days by subcutaneous injection. Antidepressive-like effects were tested three days after the last administration.
*Data shown in each group are the mean +/- SEM, n=8. **p < 0.005, ***p < 0.001*

Figure 2. ACD856 increases phosphorylation of TrkB in SH-SY5Y cells

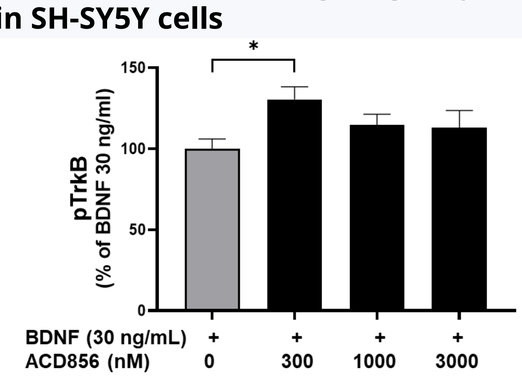

Figure 2. SHSY5Y-TrkB cells were incubated with ACD856 for 10 min. pTrkB was determined by ELISA.
*Data shown are the mean from three independent experiments including all biological replicates +/- SE, *p < 0.05 .*

Fig 5. ACD856 increases the levels of ATP in PCN's in a model of energy deprivation-induced toxicity

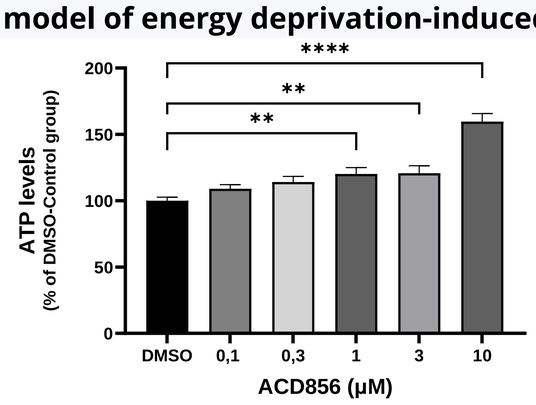

Figure 5. PCN's incubated with ACD856 in NBA-media and the ATP levels were determined by ToxGlo assay.
*Data shown are the mean +/- SEM, n=5. **p < 0.01 ****p < 0.001 vs DMSO-control values.*

Figure 7. Repeated administration of low doses of ACD856 improves memory

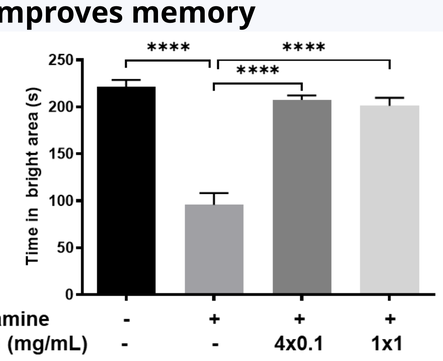

Figure 7. Animals were treated with ACD856 repeatedly for 4 days (0.1 mg/kg) or with a single subcutaneous injection (1 mg/kg) followed by scopolamine (0.3 mg/kg) 30 minutes prior to training.
*Data shown in each group is the mean +/- SEM, n=8. ****p < 0.0001*

Conclusion

We identified ACD856 as a positive allosteric modulator of Trk-receptors acting as a cognitive enhancer in vivo and demonstrate herein promising results supporting increased plasticity, neurorestorative and neuroprotective properties. The results of repeated administration of ACD856 indicate that the compound improves neuronal plasticity or in other ways increase network connectivity in regions of importance for depression and cognition.

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