CVL-354, a novel, brain penetrant and selective kappa opioid receptor antagonist

Georgette L. Suidan,[1] Galen Missig,[1] Sokhom Pin,[1] Srinivas Chakilam,[1] Sridhar Duvvuri,[1] Philip Iredale[1]

[1]Cerevel Therapeutics, Cambridge, MA.

Presenting Author: Galen Missig, galen.missig@cerevel.com

Corresponding Author: Georgette L. Suidan, georgette.suidan@cerevel.com

CONCLUSIONS

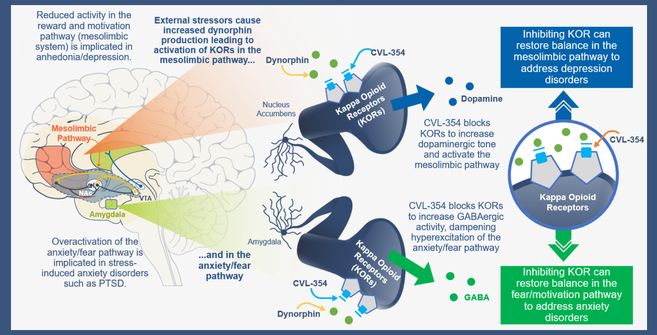
CVL-354 is a selective kappa opioid receptor (KOR) antagonist that exhibits:

- A 31-fold binding affinity to hKOR over hMOR in vitro.
- Antagonist activity at KOR (IC_{50} = 0.042 nM) with a lower potency at MOR (IC_{50} = 9.1 nM) in vitro.
- 27-fold selectivity for KOR (IC_{50} = 2.2 nM) over MOR (IC_{50} = 59.7 nM) in vivo in mouse.
- Pharmacodynamic selectivity for KOR over MOR as indicated by thermal sensitivity in the tail flick test.

CVL-354 can block KOR-mediated motivational deficits in mice as:

- Treatment dose-dependently reverses the suppression of progressive ratio responding induced by spiradoline.

Targeting kappa opioid receptors for psychiatric disorders



DISCLOSURES: GLS, GM, SP, SC, SD, PI are employees of Cerevel Therapeutics and may hold stock and/or stock options in the company.

REFERENCES: 1. Knoll AT, Meloni EG, Thomas JB, et al. Anxiolytic-like effects of kappa-opioid receptor antagonists in models of unlearned and learned fear in rats. J Pharmacol Exp Ther 2007; 323(3):838-45. 2. Van't Veer A, Carlezon WA Jr. Role of kappa-opioid receptors in stress and anxiety-related behavior. Psychopharmacology 2013; 229(3):435-452

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Abbreviations	
KOR	Kappa Opioid Receptor
MOR	Mu Opioid Receptor
DOR	Delta Opioid Receptor
ORL1	Opioid receptor-like 1
Ki	Equilibrium dissociation constant for inhibitor of ligand and receptor
IC50	Concentration of compound required for 50% inhibition
ED50	Dose of compound required for 50% efficacy
CHO	Chinese Hamster Ovary cells
SEM	Standard error of the mean

INTRODUCTION

Kappa opioid receptors (KOR) have strong activity throughout many key regions of the brain whose activity defines psychological phenomena, including motivation incentivized by reward or addictive substances, as well as anxiety[1,2]. As a regulator of both cellular excitability and synaptic transmission of key circuits involved in reward and mood, KORs present a unique therapeutic target for clinical investigation for the treatment of major depressive disorder and substance abuse disorder.

OBJECTIVE

- To characterize in vitro and in vivo binding, selectivity, and functional activity of CVL-354, a novel KOR antagonist.

METHODS

Target engagement and pharmacokinetics

- CHO cells expressing either human KOR, MOR, DOR, or ORL1 were examine for binding and functional antagonism studies.
  - Binding was determined using radioligand competition binding assay using [^{3}H]-Diprenorphine, a non-selective opioid antagonist.
  - Functional activity was evaluated using a cAMP competitive immunoassay using cAMP with an acceptor dye (D2).
- In vivo receptor occupancy was measured via administration of CVL-354 followed by administration of either [^{3}H]-GR103545 for KOR or [^{3}H]-carfentanil for MOR and measuring radioactivity in the brain.

Thermal Sensitivity

- A tail-flick unit (Ugo Basile) was used to measure withdrawal response to a thermal stimulus of mice injected (S.C.) with CVL-354 in combinations with different doses of either spiradoline or DAMGO 30 minutes prior to testing.

Progressive Ratio Response

- Single-chamber progressive ratio units (MED Associates Inc.) with three nose poke holes were used to run a progressive ratio response paradigm. In this procedure a mice must nose poke in response to a light to receive a sucrose pellet (Dustless Precision Pellets). The number of correct nose pokes required to receive a reward increased progressively throughout the session according to the function: nose pokes (rounded to the nearest integer) = $5e^{R*0.2}$-5, where R is equal to the number of rewards earned + 1. Compounds were injected 30 (S.C.) minutes prior to testing.

Statistics

- One-way ANOVA (tail flick and progressive ratio responding) were used and Dunnett's post hoc tests comparing CVL-354 doses to the control groups of either vehicle alone or spiradoline/DAMGO alone as indicated.

All procedures were carried out in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines.

RESULTS

IN VITRO BINDING AND ACTIVITY

Table 1. CVL-354 pharmacokinetics and activity in vitro

Assay		KOR	MOR
***In Vitro* Binding** (CHO-cells expressing hKOR, or hMOR)	Ki (nM)	1.47	46.2
	Replicates (n)	4	4
***In Vitro* Functional Antagonism** (CHO-cells expressing hKOR, or hMOR; cAMP endpoint)	IC50 (nM)	0.042	9.1
	Replicates (n)	4	4

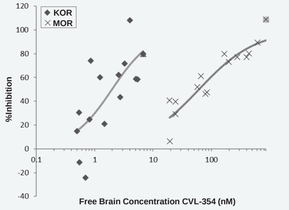
Summary of results from in vitro pharmacology study and pharmacokinetics examining CVL-354 binding to KOR and MOR.

- In vitro binding data suggests CVL-354 has a 31-fold binding affinity for hKOR over hMOR indicating selectivity for KORs.
- In vitro functional activity measuring cAMP indicated that:
  - CVL-354 is a selective antagonist for KOR, and to a much lesser extent MOR, with no detectable activity at either human DOR or human ORL-1.
  - CVL-354 did not demonstrate agonist activity at either receptor at concentrations tested (up to 1 µM).
- Pharmacokinetic studies in mouse, rat and nonhuman primate demonstrated that CVL-354 is brain penetrant and has greater brain penetration in higher species.

TARGET ENGAGEMENT

Figure 1. Unbound brain in vivo receptor occupancy selectivity of CVL-354 at KOR and MOR

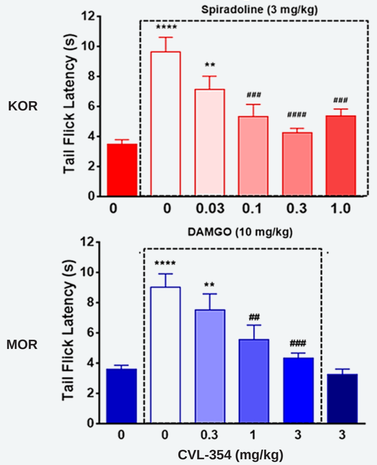
CVL-354 produced a dose dependent inhibition of [^{3}H]-GR103545 binding at KOR, and [^{3}H]-carfentanil binding at MOR. N = 4 per group, C57BL/6J mice.



- Target engagement studies suggest CVL-354 has an overall potency of 2.2 nM at KOR and a 59.7 nM at MOR resulting in a 27-fold pharmacodynamic selectivity.

THERMAL SENSITIVITY

Figure 2. CVL-354 dose-dependently reverses KOR- and MOR-induced thermal analgesia

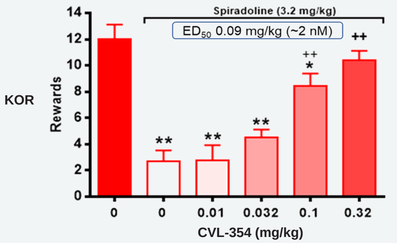


Mean tail flick latency following treatment with CVL-354 or vehicle and challenged with spiradoline or vehicle ±SEM. Symbols represented are p < (**=0.01), (***=0.001), (****=0.0001) versus vehicle; and p < (##=0.01), (####=0.0001) versus spiradoline, Dunnett's multiple comparison test. N = 10 per group, ICR male mice.

- Administration of the KOR agonist spiradoline (3 mg/kg S.C., *Top*) or the MOR agonist DAMGO (10 mg/kg S.C., *bottom*) increased tail flick latency. Co-administration of CVL-354 resulted in a dose-dependent decrease of tail flick latency, indicating reversal of KOR and MOR-mediated analgesia.
- CVL-354 reversed KOR-mediated analgesia with a minimal effective dose of 0.1 mg/kg and for MOR-mediated analgesia 1.0 mg/kg, which although qualitative, suggests a ~10-fold pharmacodynamic selectivity for KOR over MOR.

PROGRESSIVE RATIO RESPONSE

Figure 3. CVL-354 reverses KOR-mediated suppression of progression ratio responding



Mean number of rewards following treatment with CVL-354 or vehicle and challenged with spiradoline or vehicle ±SEM. Symbols represented are*, **p <0.05, 0.01, versus vehicle; ++p<0.01 versus spiradoline, Dunnett's multiple comparison test. N = 4-7 per group, C57BL/6J male mice.

- Progressive ratio responding is an assay that is sensitive to bi-directional modulation of motivation by means of nose-poking for rewards. In this assay, mice are required to nose poke in response to a light to receive reward (sucrose pellet). The number of nose pokes required to receive a reward progressively increases through a session.
- Administration of the KOR agonist spiradoline (3.2 mg/kg, S.C., 30 min prior) decreased rewards obtained indicative of a decrease in motivation for reward.
- Co-administration of CVL-354 (S.C., 30 min prior) resulted in a dose dependent attenuation of motivational suppression by spiradoline. With a significant increase from vehicle apparent at 0.32mg/kg with an ED_{50} = 0.09m/kg.