CAD-1883, a clinical-stage positive allosteric modulator of the small conductance calcium-activated potassium channel (SK channel): effects on cerebellar Purkinje neuron firing, ataxic gait, and tremor in animal models

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Introduction

Spinocerebellar ataxia (SCA) is a movement disorder caused by cerebellar dysfunction due to the irregular timing of action potentials in the olivocerebellar circuit. Small conductance calcium-activated potassium (SK) channels generate after-hyperpolarizing currents which regulate action potential timing in olivocerebellar circuit neurons. SK channels represent a molecular mechanism centrally implicated in the coordination of movement.

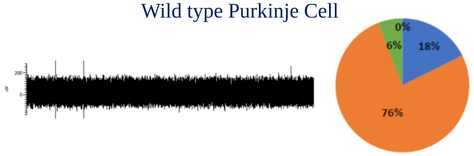
Of the three members of the SK family (SK1-3), only SK2 channels are expressed in cerebellar Purkinje neurons where they regulate tonic firing by contributing to the after-hyperpolarizing current. Inhibiting SK channels causes Purkinje cells to fire irregularly in ex vivo cerebellar slices as well as in vivo studies. Positive modulation of SK channels has been shown to decrease the firing frequency of Purkinje cells. The SK2 and SK3 channels are both also expressed in neurons of the inferior olivary nucleus which exhibits prominent SK-channel dependent after-hyperpolarizing currents.

Mechanistic evidence of the role of SK channels in regulating the pace-making activity of the principal neurons of the olivocerebellar circuit suggests the therapeutic potential of SK channel modulators in treating spinocerebellar ataxia. Mouse models of hereditary ataxia such as Episodic Ataxia 2 (EA2) "tottering" mice (which carry a loss-of-function point mutation in the CACNA1A gene encoding the P/Q-type voltage-gated calcium channel) and SCA2 58Q mice (which carry a trinucleotide poly-glutamine expansion in the ataxin-2 gene) exhibit motor deficits analogous to those observed in patients with hereditary ataxia and irregular firing of cerebellar Purkinje neurons in brain slices.

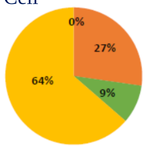
The objective of Cadent Therapeutics' lead drug discovery program has been to optimize chemical matter of positive allosteric modulation of the SK channels. Of the hundreds of analogs which have been profiled, CAD-1883 demonstrated the most exceptional properties through all studies and rapidly emerged as the development candidate due to its high SK potency, improved stability, reduced off-target liabilities, high cross-species oral bioavailability, and exquisite efficacy in a variety of in vivo and ex vivo models of ataxia and tremor.

Irregular in vivo firing of Purkinje cells in SCA 58Q mice

Wild type Purkinje Cell



Ataxic SCA2 58Q Purkinje Cell



In vivo cellular recording of Purkinje cell activity in mice in wild type (top) and SCA2 58Q (bottom) 12-month-old mice. Purkinje cells were identified by complex spike occurrence and recordings lasted for ≥5 minutes. Bursting Purkinje cells were found exclusively in the SCA2 58Q mice (right, CV = coefficient of variation).

- **Tonic** (CV < 0.15)
- **Irregular tonic** (0.15 ≤ CV ≤ 0.50)
- **Irregular** (CV > 0.50)
- **Bursting** (Clearly separated bursts or >5% of ISI outside 3 SD from mean)

CAD-1883: a potent and selective positive allosteric modulator of SK channels

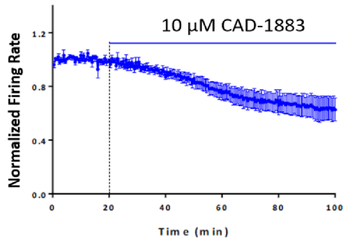
Channel	Assay	CAD-1883
hSK2	SC_{100} (μM)	0.54 ± 0.26 μM
	EC_{50} (μM)	1.97 ± 0.41 μM
hSK3	SC_{100} (μM)	0.080 ± 0.018 μM
	EC_{50} (μM)	0.82 ± 0.07 μM
hSK1	SC_{100} (μM)	> 10 μM
hIK		> 10 μM
hBK		> 10 μM

The potency and selectivity of CAD-1883 was evaluated in whole-cell recordings of HEK293 cells in which individual ion channels are over-expressed. The potency of CAD-1883 was first profiled on recombinant human SK2 and SK3 channels and it was found to be an exceptionally potent positive allosteric modulator of both channels. CAD-1883 was counter-screened against related Ca^{2+}-activated potassium channels, such as the intermediate conductance potassium channel (IK) and big conductance potassium channel (BK), which have widespread peripheral distribution and could lead to undesired side effects upon modulation. CAD-1883 was found to not modulate the SK1, IK, and BK channels. These data indicate that CAD-1883 is a highly-selective positive allosteric modulator of the SK2 and SK3 channels.

SC_{100} = Compound concentration that increases the basal current by 100%
EC_{50} = Compound concentration causing half-maximal increase in current

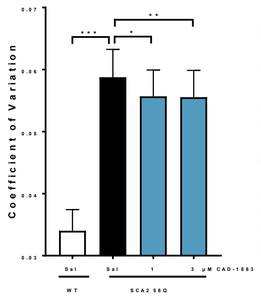
Effects of CAD-1883 on cerebellar slice electrophysiology

Reduction of wild-type mouse cerebellar Purkinje neuron firing rate with CAD-1883



Experiments with isolated Purkinje neurons in the presence of synaptic blockers (glutamate NMDA and GABA receptors) have been designed to assess intrinsic cerebellar Purkinje firing. Sagittal cerebellar slices at 37 °C were prepared from 2-3 week old C57Bl/6 wild-type mice. Changes in firing frequency were recorded for 80 minutes following application using multielectrode array. CAD-1883 reduced the firing rate of Purkinje cells by approximately 40% (n=24) which is consistent with the proposed therapeutic mechanism of positive allosteric modulation of SK channels.

Regularization of SCA2 58Q mouse cerebellar Purkinje neuron firing with CAD-1883



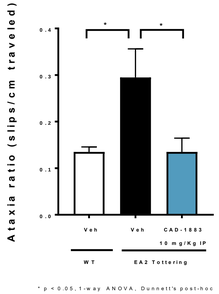
*** P<0.005, un-paired T-test
*, ** P<0.05, P<0.01, 1-way repeated-measures ANOVA, Dunnett's post-hoc

In cerebellar slices from SCA2 58Q mice, Purkinje neurons exhibit chaotic firing and a measurable increase in the coefficient of variation of the interspike interval (ISI CV), a measure of the regularity of the firing interval between action potentials. The difference in coefficient of variation of the interspike interval between wild-type (N=8) and SCA2 58Q (N=11) mice is illustrated.

Sequential bath application of 1 (N=11) or 3 μM (N=10) CAD-1883 partially reversed the increase in ISI CV observed in cerebellar slices from eleven-month old SCA2 58Q mice. These data indicate that CAD-1883 regularizes Purkinje firing by partially restoring the interspike interval in this mouse model of spinocerebellar ataxia.

Effects of CAD-1883 on mouse and rat motor dysfunction in disease models

Improvement in coordination in Episodic Ataxia 2 (EA2) mouse model with CAD-1883

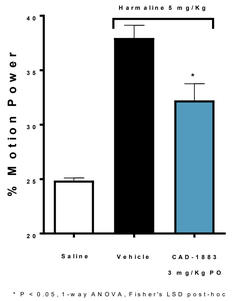


* p < 0.05, 1-way ANOVA, Dunnett's post-hoc

The ataxic symptoms in episodic ataxia 2 (EA2) "tottering" mice arise from irregularity in Purkinje cell firing due to a loss-of-function mutation in P/Q Ca^{2+} channels. In this study, EA2 mice were assessed in a parallel rod floor apparatus which counts the number of foot slips relative to the total distance traveled to define an ataxia ratio (a higher value is indicative of worsened ataxia symptoms). Eighteen 8-10 month old EA2 mice were injected intraperitoneally with CAD-1883 or vehicle 30 minutes prior to being placed in the apparatus.

Animals were assessed in a cross-over study design. At the dose administered in this study, CAD-1883 fully reversed the increase in ataxia ratio observed in EA2 vs wild-type mice. These data indicate that CAD-1883 restores normal performance in this measure of motor function in a model of hereditary cerebellar ataxia.

Reduction in tremor in rat harmaline model of tremor with CAD-1883



* P < 0.05, 1-way ANOVA, Fisher's LSD post-hoc

Rats were pre-treated with 3 mg/kg PO CAD-1883 30 minutes prior to intraperitoneal administration of 5 mg/kg harmaline, a tremor-inducing chemical. Tremor was measured for 30 minutes following harmaline administration and data were analyzed by fast Fourier transform and reported as a frequency power spectrum. Harmaline induced a significant increase in the power spectrum in a band of frequencies between 10 and 14 Hz. A dose of 3 mg/kg PO significantly reduced tremor.

Data were further analyzed by calculating the Percent Motion Power defined as the power in the 9 – 13 Hz band divided by the total power across the spectrum (0 – 30 Hz) multiplied by 100. By this analysis, 3 mg/kg PO CAD-1883 significantly reduced harmaline-induced tremor (N=13 for harmaline + vehicle, N=8 for harmaline + 3 mg/kg CAD-1883).

Conclusion

CAD-1883 is a selective, positive allosteric modulator of the SK channel that modulates Purkinje firing in wild-type mouse cerebellar slices, regularizes chaotic Purkinje neuron firing in cerebellar slices from SCA2 58Q mice, reduces ataxic symptoms in EA2 mice, and reduces tremor in the rat harmaline model of essential tremor. CAD-1883 is currently being evaluated in Phase 1 clinical studies.

References

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Lay Summary

Cadent Therapeutics is developing CAD-1883 as a potential treatment for spinocerebellar ataxia and related movement disorders. CAD-1883 is an investigational drug which is taken by mouth and the drug travels through the body to the brain where it elicits its biological effect. Pre-clinical studies in ataxic animals, which have the same underlying genetic signature of spinocerebellar ataxia as humans, have shown that the cells in the brains of these animals are firing irregularly. CAD-1883 interacts directly with channels in the brain which are responsible for controlling this firing, and the interaction between CAD-1883 and these channels is expected to restore regular firing. Improvement in ataxic gait with CAD-1883 has been successfully demonstrated in animal models. Restoration of the normal cadence of cellular firing is anticipated to improve the motor coordination of people with spinocerebellar ataxia as well. CAD-1883 is currently being assessed in a clinical trial with healthy human volunteers to determine safe drug levels. Cadent Therapeutics plans to initiate a clinical trial with CAD-1883 in patients with spinocerebellar ataxia in early 2019. For more information about this trial, please visit www.cadenttx.com.