AUT00206, a Kv3 channel modulator with potential for the treatment of Fragile X Syndrome

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Introduction

The Fragile X mental retardation protein (FMRP) regulates the expression of Kv3.1 ion channels[1], which may contribute to symptoms of FXS, including hypersensitivity to sound and cognitive deficits. Furthermore, parvalbumin-positive GABA interneurons, which specifically express Kv3 channels[2], are implicated in the neuropathology of FXS[3,4].

Sign, Symptom or Morbidity	M	F
Developmental Delay or Intellectual Disability	96%	64%
Attention Problems	84%	67%
Anxiety	70%	56%
Hyperactivity	66%	30%
Autism	46%	16%
Self Injury	41%	10%
Aggressiveness	38%	14%
Seizures	18%	7%
Depression	12%	22%

Data from the Center for Disease Control and Prevention, US.

FMRP is predominantly expressed in neurons and interacts with the coding region of mRNA transcripts encoding pre- and postsynaptic proteins. FMRP level has a profound effect on higher brain function. FMRP regulates the expression and function of a wide range of neuronal proteins, with complex consequences. FMRP has been shown to regulate the expression and function of Kv3.1 voltage-gated potassium channels. For example, altered expression of Kv3.1 channels by auditory neurons may contribute to hypersensitivity to sound that is a common symptom in FXS.

Methods

Fmr1 KO2 mice (on a C57Bl6 background, back-crossed at least 8 times) were used in the study, 10 mice per group. Mice were 2 months of age at the start of dosing.

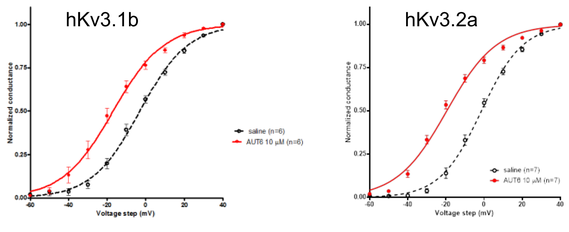
AUT00206 was dosed daily at 30 or 60 mg/kg i.p. for 6 weeks prior to testing. No adverse effects of the drug were noted in the appearance of the animals. Behavioural tests were conducted 30 minutes after the final dose of AUT00206.

Vehicle (V): Captisol (12.5%), 0.5% HPMC, 0.5 % Tween80 in water

In an earlier experiment, a different cohort of mice were dosed for 15 days at 30 mg/kg i.p. only. This earlier experiment included the Nesting and Fear Conditioning tests shown here. Other tests in this earlier study found similar results to those shown here for locomotor activity, marble burying and audiogenic seizures.

AUT00206 Modulates Kv3 Channels

AUT00206 produced a concentration-dependent leftward shift of the voltage-dependence of activation of hKv3.1b and hKv3.2 channels:

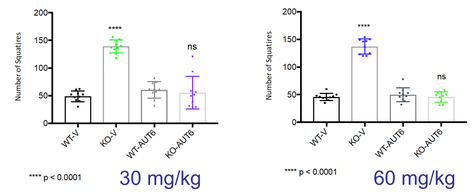


AUT00206	hKv3.1b	hKv3.2a
10 µM	- 7.1 mV	- 11.2 mV
30 µM	- 15.9 mV	- 29.2 mV

Voltage shifts are corrected for the effect of vehicle (0.1% DMSO)

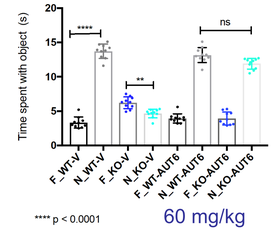
AUT00206	pEC50	Max. Potentiation
Kv3.1b	5.25±0.05 (n=8)	131±11 % (n=8)
Kv3.2a	5.68±0.05 (n=8)	129±9 % (n=8)

Hyperactivity



FXS patients are often hyperactive. Fmr1-KO2 mice displayed significantly increased locomotor activity compared to WT mice. Activity in Fmr1-KO2 mice treated with AUT00206 was reduced to WT levels.

Cognition



Fmr1-KO2 mice showed impaired performance of the Novel Object Recognition task. This behaviour was normalised by treatment with AUT00206.

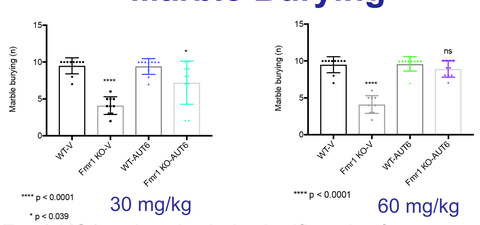
Audiogenic Seizures

	Wild running	Seizure	Death	Incidence
WT-Veh	0/10	0/10	0/10	0%
Fmr1 KO-Veh	10/10	9/10	8/10	100%
WT-AUT6 (30)	0/10	0/10	0/10	0%
Fmr1 KO-AUT6 (30)	6/10	5/10	5/10	60%

	Wild running	Seizure	Death	Incidence
WT-Veh	0/10	0/10	0/10	0%
Fmr1 KO-Veh	9/10	8/10	7/10	90%
WT-AUT6 (60)	0/10	0/10	0/10	0%
Fmr1 KO-AUT6 (60)	3/10	3/10	3/10	30%

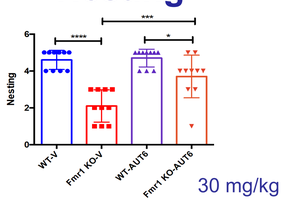
Fmr1-KO2 mice are sensitive to seizure induced by loud sound. Treatment with AUT00206 reduced the incidence of seizure in a dose-dependent manner.

Marble Burying



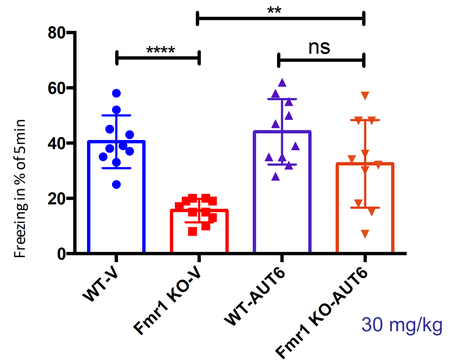
Fmr1-KO2 mice buried significantly fewer marbles, reflecting a disruption of this ethological behaviour. AUT00206 treated Fmr1-KO2 mice showed increased marble burying similar to WT mice.

Nesting



Fmr1 KO2 mice showed a significant reduction in nest building quality compared to WT mice. AUT00206 significantly improved the quality of nest building of the Fmr1 KO2 mice.

Fear Conditioning



Fmr1-KO2 mice displayed significantly reduced freezing, a measure of fear-related memory. Treatment with AUT00206 normalised the freezing behaviour in most animals.

Summary and Conclusions

In the present study, AUT00206 reversed partially or fully each of the abnormal behaviours observed in Fmr1 KO2 mice. Importantly, the drug did not affect the behaviour of wild type mice, suggesting that effects seen in the Fmr1 KO2 mice were not due to a non-specific central effect such as sedation. Indeed, the drug produced positive effects on behaviours such as marble burying, nesting, and novel object recognition which could not be explained by a CNS depressant effect.

Doses of 30 and 60 mg/kg of AUT00206 were effective in this study, with the higher dose showing greater effect in several of the tests. It will be important to confirm that drug concentrations associated with these effects can be achieved in humans.

AUT00206 is a positive modulator of Kv3.1 and Kv3.2 channels, which is currently in clinical development for the treatment of schizophrenia. Autifony has received Orphan Drug Designation for AUT00206 for the treatment of Fragile X Syndrome from the FDA.

The results of this study support the consideration of AUT00206 and Kv3.1/3.2 modulators in the treatment of Fragile X Syndrome.

References

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