Fenfluramine in the Treatment of Drug-Resistant Seizures: *Back-Translation* Using Zebrafish and Mice

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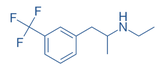
INTRODUCTION



Epilepsy

- Common neurological disease (up to 75 million people worldwide)
- 30% not responding to current anti-epileptic drugs (AEDs) (i.e. drug-resistant)
- Etiology: genetic, structural, metabolic, infectious/inflammatory or unknown
  - *SCN1A* (*neural sodium channel, type 1, subunit α*): most prominent epilepsy gene
  - *SCN1A* mutation in 80% of Dravet syndrome (DS) patients[1]
- DS is a rare, severe and **drug-resistant** epilepsy syndrome

Fenfluramine

- Fenfluramine (FFA) is a serotonergic agent, though other pathways can be involved[2]
- Clinical data emphasize its successful use in treating **drug-resistant** seizures in DS patients[3]
- Efficacy of FFA as an AED to treat **other** seizures or epilepsy syndromes is currently unknown

Animal models of (drug-resistant) seizures

- Genetic zebrafish (ZF) model of DS: *scn1Lab*$^{-/-}$ mutant ZF larvae mimic the **drug-resistant** seizures, seen in DS patients[4]
- Chemical ZF model of generalized motor seizures: wild type (WT) ZF larvae treated with the proconvulsant, pentylenetetrazole (PTZ), a $GABA_A$-antagonist. Some AEDs are not able to reduce PTZ-induced seizures, which leads to a **limited** number of **effective AEDs** in this model (6/10, i.e. 60%).[5]
- Electrical mouse model (6-Hz): potential drug screening platform for **drug-resistant** seizures when the intensity is set on 44 mA. This idea is based on the fact that 44 mA 6-Hz seizures are resistant to several AEDs (compared to 22 mA).[6,7]

RATIONALE

Does fenfluramine significantly reduce (drug-resistant) seizures?

- In a ZF **genetic** model of DS (*scn1Lab*$^{-/-}$ mutant ZF)?
- In a ZF **chemical** model of generalized seizures (PTZ)?
- In a mouse model of **electrically** induced seizures (6-Hz)?

METHODS

1) Zebrafish models of seizures and epilepsy

Seizures?

- Chemical model: PTZ-treated WT ZF larvae (AB strain)
- Genetic model: homozygous *scn1Lab*$^{-/-}$ mutant ZF larvae (*didy*^{s552})

Treatment?

- Larvae were immersed in aqueous solutions (one 6 dpf larva per well of a 96-well plate): Vehicle, VHC (dimethyl sulfoxide, DMSO 0.1%) or FFA (25, 50 or 100 µM)
- 18-24 hours (h) treatment (8-12 larvae per condition; in duplicate or triplicate)

Behavior (locomotor) *7 dpf*

- Lardist: 10 minutes (min) after 30 min habituation, a surrogate marker for the epileptiform behavior
- Automated tracking device (ZebraBox™ apparatus; Viewpoint, Lyon, France)
- A statistically significant decrease in epileptiform behavior (compared to VHC)
- Statistics: One-way ANOVA followed by Dunnett's multiple comparison tests

Brain activity *7 dpf*

- Non-invasive forebrain open-field recordings for 10 min
- ZF larva embedded in 2% low-melting-point agarose (Digidata® 1440A digitizer; Axon Instruments, USA)
- To confirm anticonvulsant effects of FFA treatment, if indicated by the behavioral assays (compared to VHC)
- Statistics: Mann-Whitney U tests

2) Mice model of seizures

Seizures?

- Electrically induced in NMRI mice (30-35 g): corneal stimulation (6-Hz, 0.2 ms pulse width, 44 mA)[6]

Treatment?

- Intraperitoneal (IP) injection: VHC (50/50 DMSO/PEG200) or FFA (5 mg/kg and 20 mg/kg)
- 1-h treatment a priori (5-6 mice per condition) + 0.5% xylocaine drop on eyes right before seizure induction

Behavior

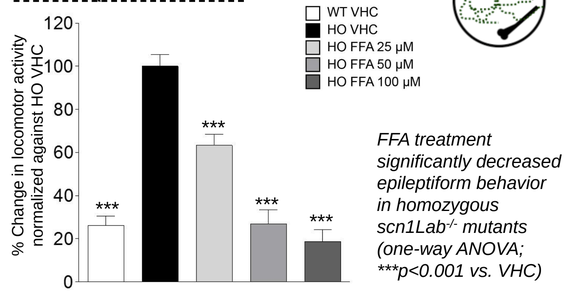
- Plexiglas cage; analysis: stun, forelimb clonus, twitching of vibrissae, and Straub tail
- Statistics: Protection analyses by Fisher's exact tests and seizure duration by one-way ANOVA followed by Dunnett's multiple comparison tests

RESULTS

1) Zebrafish

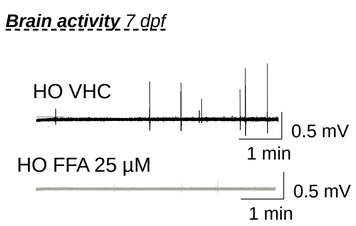
scn1Lab$^{-/-}$ *mutant ZF*

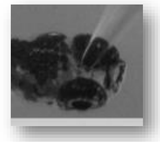
Behavior (locomotor) *7 dpf*



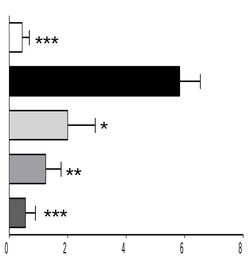
*FFA treatment significantly decreased epileptiform behavior in homozygous scn1Lab$^{-/-}$ mutants (one-way ANOVA; ***p<0.001 vs. VHC)*

Brain activity *7 dpf*

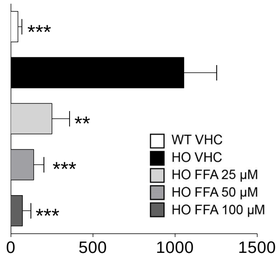




We confirmed a concentration-dependent anti-epileptiform activity by LFP recordings (Mann-Whitney U test; p<0.05 vs. VHC)



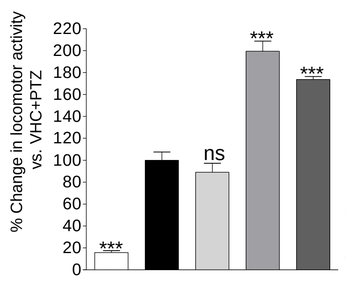
Frequency of epileptiform events (/10 min recording)



Mean cumulative duration of epileptiform event (msec/10 min recording)

Chemically induced seizures in ZF (PTZ)

Behavior (locomotor) *7 dpf*

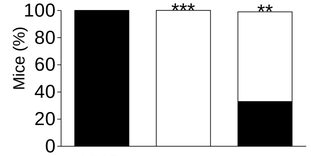


There were no significant antiseizure effects observed by FFA treatment in the PTZ-induced ZF model of seizures (one-way ANOVA; p>0.05 vs. VHC+PTZ)

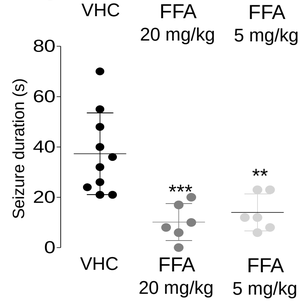
2) Mice

Electrically induced seizures: mice (6-Hz)

FFA treatment significantly decreased the duration of seizures in 6-Hz mice (Mann-Whitney U test; p<0.05 vs. VHC). FFA (5 and 20 mg/kg, n=6 for both groups), compared to VHC-treated (n=10).



Protection: defined as the absence of a seizure within the expected time frame (i.e. below the minimum seizure duration in VHC-treated mice)



Figures represent statistically significant differences by (*) if p<0.05, (**) if p<0.01 and (***) if p<0.001.

CONCLUSIONS

- *The efficacy of FFA was confirmed in the ZF model of DS and is in line with the clinical efficacy of FFA in treating drug-resistant seizures in DS patients.*
- *FFA was not active in a widely used ZF model of seizures induced by PTZ, an antagonist of the $GABA_A$ R, and known to be most sensitive to GABAergic AEDs. However, the exact effects of FFA on GABAergic neurotransmission need to be explored.*
- *FFA significantly reduced seizures in the mouse 6-Hz model, which demonstrated to be a model for drug-resistant seizures.*

Efficacy of FFA beyond the treatment of DS ? → ***FFA's efficacy in treating drug-resistant seizures in other epilepsies should be further explored.***

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2. Sourbron, J. et al. Front. Pharmacol. 8, 191 (2017)
3. https://globenewswire.com/news-release/2017/04/27/973531/0/en/Zogenix-Completes-Enrollment-in-First-ZX008-Phase-3-Clinical-Trial-in-Dravet-Syndrome.html
4. Baraban, S.C., Dinday, M.T. & Hortopan, G A. Nat. Commun. 4: 2410 (2013)
5. Leclercq, K. et al. Epilepsy Behav. 45, 53–63 (2015)
6. Leclercq, K., Matagne, A. & Kaminski R.M. Epilepsy Res. 108, 675–683 (2014)
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