**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)
- Genetics: genetic, structural, metabolic, infectious/inflammatory or unknown
  - SCN1A (neuronal sodium channel, type 1, subunit α): most prominent epilepsy gene
  - SCN1A mutation in 80% of Dravet syndrome (DS) patients
- DS is a rare, severe and drug-resistant epilepsy syndrome

**Fenfluramine**
- Fenfluramine (FFA) is a serotonergic agent, though other pathways can be involved
- Clinical data emphasize its successful use in treating drug-resistant seizures in DS patients
- 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<sup>−/−</sup> mutant ZF larvae mimic the drug-resistant seizures, seen in DS patients
- Chemical ZF model of generalized motor seizures: wild type (WT) ZF larvae treated with the proconvulsant, pentylenetetrazole (PTZ), a GABA<sub>A</sub> antagonist. Some AEDs are not able to reduce PTZ-induced seizures, which leads to a limited number of effective AEDs in this model (8:10, i.e., 60%).
- 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).

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**METHODS**

**1) Zebrafish models of seizures and epilepsy**

**Seizures**
- Chemical model: PTZ-treated WT ZF larvae (AB strain)
- Genetic model: homozygous scn1Lab<sup>−/−</sup> mutant ZF larvae (αdy<sup>−/−</sup>)

**Treatment**
- Larvae were immersed in aqueous solutions (one 6 dpi 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**
- Locomotion: 7 dpi
  - Larval: 10 minutes (min) after 30 min habituation, a surrogate marker for the epileptiform behavior
  - Automated tracking device (ZebraBox™, Viewpoint, Lyon, France)
- A statistically significant decrease in epileptiform behavior (compared to VHC)

**Statistics**
- One-way ANOVA followed by Dunnett’s multiple comparison tests

**2) Mice model of seizures**

**Seizures**
- Electrically induced seizures: mice (6-Hz)

**Treatment**
- FA 50 µM + PTZ

**Behavior**
- Locomotion: 7 dpi

**Statistics**
- One-way ANOVA followed by Dunnett’s multiple comparison tests

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**RESULTS**

**Chemically induced seizures in ZF (PTZ)****

**Behavior (locomotion), 7 dpi**

**Fenfluramine (FAA) was not active in a widely used ZF model of seizures induced by PTZ, an antagonist of the GABA<sub>A</sub> receptor. Some AEDs are not able to reduce PTZ-induced seizures, which leads to a limited number of effective AEDs in this model (8:10, i.e., 60%).**

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**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<sub>A</sub> receptor, and known to be most sensitive to GABA<sub>A</sub>ergic AEDs. However, the exact effects of FFA on GABA<sub>A</sub>ergic 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.
- FFA’s efficacy in treating drug-resistant seizures in other epilepsies should be further explored.

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**REFERENCES**