Antiepileptic effect of the enantiomers of fenfluramine and norfenfluramine in a Dravet zebrafish model

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INTRODUCTION

Dravet Syndrome

A severe, severe, and drug-resistant epilepsy syndrome that typically presents as a developmental epileptic encephalopathy in infancy

• SCN1A (neural sodium channel, type 1, subunit a) mutation found in 80% of patients with Dravet syndrome (DS)
• Treatment involves combination therapy with multiple antiepileptic drugs (AEDs), though patients continue to have seizures (45% of patients experience 24 tonic-clonic seizures per month)⁴. Hence, there is a need for new and improved AEDs

Fenfluramine and Norfenfluramine

Fenfluramine (FFA) is observed in clinical trials to be efficacious and safe in seizure reduction as an add-on therapy for patients with DS.⁵ FFA is a racemic mixture and it is currently unknown whether its antiepileptic activity is due to either one or both of its enantiomers⁶.

Norfenfluramine (norFFA) is a principal (N-dealkylated) metabolite of FFA and is reported to have similar neurobiological activity.⁷ The mechanisms of action of norFFA could be important for the antiepileptic effects of FFA. Therefore, it is of interest to understand whether the enantiomers of norFFA have antiepileptic activity

Zebrafish

• Established scn1Lab⁻/- mutant zebrafish model recapitulates DS, showing drug-resistant seizures and epileptiform brain activity, and is an appropriate model for translation of pharmacological findings⁸

RATIONALE

The antiepileptic activity of the enantiomers of FFA and norFFA has not been investigated to date but is of particular interest considering the clinical efficacy and safety of low-dose (±)FFA. Hence, we investigated the antiseizure and antiepileptiform activity of (+)FFA, (-)FFA, (+)norFFA, and (-)norFFA in the established genetic zebrafish model of DS, the scn1Lab⁻/- mutant model, to determine the active enantiomers and their efficacy

METHODS

Treatment

• Wildtype (WT) and scn1Lab⁻/- larvae at 6 days post-fertilization (dpf) were exposed to vehicle (VHC, 0.1% DMSO), (+)FFA, (-)FFA, (+)norFFA, or (-)norFFA by water immersion for 22 to 24 hours of incubation, followed by safety or activity assessment

Safety Assessment

• Maximum tolerated concentration (MTC) of each compound was determined, and the lowest MTC, ie, 50 µM for (-)FFA, was used as the test concentration for all compounds

• MTC was defined as the highest concentration at which no larvae (n = 12) died or showed signs of toxicity or locomotor impairment

Behavioral Antiseizure Activity Analysis

• Automated tracking (n = 21-25, ZebraBox, Viewpoint)
• 30-min habituation
• 10-min quantification of Lardest (distance traveled in large movements), a surrogate marker for seizure behavior
• Statistics: one-way ANOVA followed by Dunnett’s multiple comparison test, mean±SEM

Electrographical Antiepileptiform Activity Analysis

• 10-min non-invasive local field potential recordings from the optic tectum (n = 10-18)
• Larvae embedded in 2% low-melting point agarose
• 10-min non-invasive local field potential recordings from the optic tectum (n = 10-18)
• Larvae embedded in 2% low-melting point agarose
• 10-min quantification of Lardist (distance traveled in large movements), a surrogate marker for seizure behavior
• Statistics: one-way ANOVA followed by Dunnett’s multiple comparison test, mean±SEM

RESULTS

Antiseizure Activity of the Enantiomers of Fenfluramine and Norfenfluramine

• Both enantiomers of FFA show antiseizure activity, suggesting that both contribute to the activity of (±)FFA. The efficacy profiles of (+)- and (-)-FFA in comparison to that previously tested for (±)-FFA suggest an additive effect of the enantiomers. (+)-FFA is more efficacious than (-)-FFA, ie, 84% vs 41% reduction in seizure behavior

• The enantiomers of norFFA also demonstrate antiseizure activity, suggesting that their activity contributes to that of (±)-FFA. In line with the findings for FFA, (+)-norFFA is more efficacious than (-)-norFFA, ie, 80% vs 45% reduction in seizure behavior

Antiepileptiform Activity of the Enantiomers of Fenfluramine and Norfenfluramine

• In line with the behavioral results, both enantiomers of FFA demonstrate antiepileptiform activity, suggesting that both contribute to that of (+)-FFA. The efficacy of (+)- and (-)-FFA are comparable

• In line with the behavioral results, the enantiomers of norFFA also demonstrate antiepileptiform activity, suggesting that their activity contributes to that of (±)-FFA. However, (+)- and (-)-norFFA are less efficacious than (+)- and (-)-norFFA, respectively

CONCLUSIONS

The antiepileptic effect of the enantiomers of FFA and norFFA was determined for the first time using the established genetic zebrafish model of DS. All enantiomers significantly ameliorated behavioral seizures and electrographic epileptiform discharges, thereby demonstrating their antiseizure and antiepileptiform activity and suggesting their involvement in the antiepileptic effects of FFA seen in the clinic

The efficacy profiles of (+)- and (-)-FFA in comparison to that of (±)-FFA, previously tested, suggest an additive effect

Interestingly, the antiseizure activity of (+)-FFA is more efficacious than that of (-)-FFA. In line with these findings, the antiseizure activity of (+)-norFFA is also more efficacious than that of (-)-norFFA

REFERENCES
