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BRIEF COMMUNICATION

Epilepsia®

Drug repurposing for Dravet syndrome in *scn1Lab*$^{-/-}$ mutant zebrafish

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Summary

Dravet syndrome (DS) is a severe genetic epileptic encephalopathy with onset during the first year of life. Zebrafish models recapitulating human diseases are often used as drug discovery platforms, but also for drug repurposing testing. It was recently shown that pharmacological modulation of three serotonergic (5-HT) receptors (5-HT_{1D}, 5-HT_{2C}, 5-HT_{2A}) exerts antiseizure effects in a zebrafish *scn1Lab*$^{-/-}$ mutant model of DS. Using the zebrafish DS model, our aim was to examine the possibility of repurposing efavirenz (EFA), lisuride (LIS), and rizatriptan (RIZA), marketed medicines with a 5-HT on- or off-target profile, as antiepileptic drugs for DS. To examine whether these compounds have a broader antiseizure profile, they were tested in pentylenetetrazol and ethyl ketopentenoate (EKP) zebrafish models. Pharmacological effects were assessed by locomotor behavior, local field potential brain recordings, and bioluminescence. EFA was active in all models, whereas LIS was selectively active in the zebrafish DS model. Mainly, a poor response was observed to RIZA. Taken together, our preclinical results show that LIS could be a potential candidate for DS treatment. EFA was also active in the EKP model, characterized by a high level of treatment resistance, and hence these data are potentially important for future treatment of drug-resistant epilepsy.

KEYWORDS

drug-resistant, epilepsy, marketed medicines

1 | INTRODUCTION

With the aim to bring novel therapeutics to the market together with reducing the costs associated with traditional de novo drug development, many efforts are underway to repurpose existing drugs.[1]

Dravet syndrome (DS) is a rare, severe genetic epileptic encephalopathy with onset during the first year of life in an otherwise healthy child, later accompanied by intellectual disability. DS has always been considered as one of the most pharmacoresistant epilepsy syndromes with a high unmet medical need. Importantly, low-dose fenfluramine added to patients' antiepileptic drug (AED) regimen was found recently to be highly efficacious in reducing seizure frequency during the treatment period.[2]

Zebrafish represent an in vivo model that can mirror well a large variety of human diseases with a high predictive validity.[3] We and others have previously demonstrated effective inhibition by fenfluramine of seizurelike locomotor activity and epileptiform discharges in a zebrafish *scn1Lab*$^{-/-}$ mutant model of DS.[4,5] Zebrafish-based models are also amenable to fast screening of large libraries in a

target-unbiased phenotypic way. As a consequence, they are well fitted to function as discovery platforms for drug repurposing testing,[6] for example, in the search for new therapeutic candidates to treat DS patients and possibly other drug-resistant epilepsies.[5]

Using the zebrafish *scn1Lab*$^{-/-}$ mutant model of DS, recently the role of serotonergic (5-HT) modulation in treating drug-resistant seizures was underlined.[4,5] Hence, we performed a literature search on marketed medicines with a 5-HT on- or off-target profile and found three compounds, rizatriptan (RIZA),[7] lisuride (LIS),[8] and efavirenz (EFA),[9] that have not been examined before in any preclinical DS model.

RIZA[7] is one of the triptan derivatives that are used for the treatment of migraine headaches. LIS[8] is an ergot derivative that acts as an antiparkinson drug, and EFA is a nonnucleoside reverse transcriptase inhibitor used to treat human immunodeficiency virus type 1.[9] Of interest, LIS has been used successfully before to treat cortical reflex myoclonus in patients[10] and was also active in some rodent seizure models.[11]

Using the zebrafish DS model, our aim was to examine the possibility to repurpose these marketed medicines as AEDs, in particular in difficult to treat epilepsies such as DS. To examine further their potential broader antiseizure profile, we also tested the compounds in a pentylenetetrazol (PTZ)[12] and a treatment-resistant ethyl ketopentenoate (EKP) model[13] in zebrafish. Our results show that LIS selectively decreased seizure activity in the DS zebrafish model, whereas EFA exhibited a broader antiseizure activity. Overall, RIZA was inactive.

2 | MATERIALS AND METHODS

2.1 | Zebrafish maintenance and experimental setup

Zebrafish experiments were approved by the Ethics Committee of the University of Leuven (approval number 154/2015 and P101/2010) and by the Belgian Federal Department of Public Health, Food Safety, and Environment (approval number LA1210199). All procedures were carried out following the Declaration of Helsinki and according to the European Community Council directives 86/609/EEC. Husbandry and genotyping (*scn1Lab* mutants) of zebrafish was performed as described previously.[4] Wild-type zebrafish (AB strain) were used for experiments with PTZ and EKP. The *Tg(elavl3:eGFP-apoAequorin)* zebrafish line was used for the bioluminescence experiments.[13]

2.2 | Pharmacological evaluation in zebrafish

Compounds were purchased from Tocris. (±)Fenfluramine was a gift from Prof. B. Ceulemans (Antwerp, Belgium). Stock solutions were prepared in DMSO and kept at −20°C. Prior to experimental work, stock solutions were further diluted in embryo medium (Danieaus or E3 medium). In all experiments, the final DMSO concentration was 0.1% vol/vol. Zebrafish larvae were pretreated at 6 days postfertilization (dpf) for 22 hours with 0.1% DMSO (vehicle control [VHC]) or compound (at its maximum tolerated concentration [MTC], determined as before[4]). In the case of LIS, lower concentrations were also tested. Total locomotor activity as well as the forebrain local field potential (LFP) recordings were performed at 7 dpf, as reported previously by our group.[4] Similarly, *Tg(elavl3:eGFP-apoAequorin)* zebrafish larvae were preexposed to coelenterazine-h (NanoLight Technology) and pretreated at 6 dpf with VHC or compound. Emitted photons were counted in a light-tight thermostatted perfusion chamber at 7 dpf as described previously.[13] For chemical induction of seizures, larvae were acutely exposed after compound pretreatment to 20 mmol·L^{-1} PTZ[12] or 400 μmol·L^{-1} EKP.[13]

2.3 | Statistical analysis

The larval locomotor activity was examined by unpaired *t* tests or one-way analysis of variance and subsequent Dunnett multiple comparison tests.[4] Electrographic brain activities and bioluminescence data were analyzed by using Mann-Whitney *U* tests.[4]

3 | RESULTS

We examined the effects of EFA, LIS, and RIZA at their MTCs (ie, 3.12, 0.10, and 100 μmol·L^{-1}, respectively), in a homozygous *scn1Lab*$^{-/-}$ mutant zebrafish model of DS and in two chemically induced seizure models (PTZ and EKP).

There was a significant decrease of epileptiform locomotor activity in the *scn1Lab*$^{-/-}$ mutants treated with LIS (Figure 1A), with a clear concentration-responsiveness relationship (Figure S1). This compound, however, was inactive in the chemically induced seizure models (Figure 1B and 1C). EFA significantly decreased the seizurelike behavior in the homozygous *scn1Lab*$^{-/-}$ mutants (Figure 1A) and in the chemically induced seizure models (Figure 1B and 1C). RIZA did not reduce locomotor activity in homozygous *scn1Lab*$^{-/-}$ mutants (Figure 1A) but showed antiseizure activity in the chemical models (Figure 1B and 1C).

In line with these results, EFA and LIS were able to decrease the epileptiform brain discharges in the forebrain of *scn1Lab*$^{-/-}$ mutants, whereas RIZA was inactive (Figure 1D). In case of PTZ- and EKP-induced epileptiform

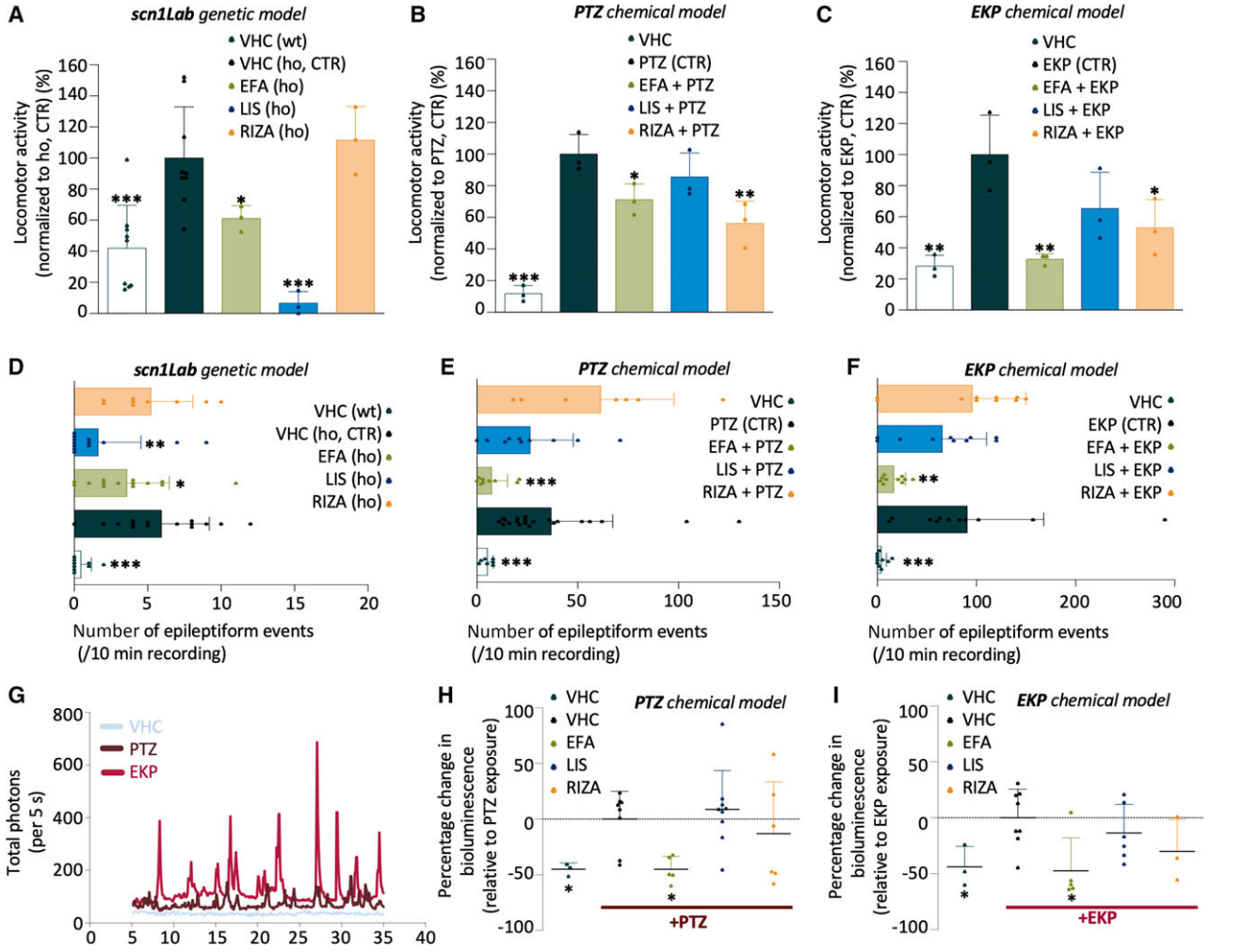

FIGURE 1 Activity profiles of efavirenz (EFA; green bars), lisuride (LIS; blue bars), and rizatriptan (RIZA; orange bars) at their maximum tolerated concentrations (ie, 3.12, 0.10, and 100 µmol·L^{-1}, respectively) as shown by scatter plots with bars. Data of vehicle-treated (VHC) wild-type (wt) larvae are represented by white bars. Data of vehicle-treated homozygous (ho) *scn1Lab*$^{-/-}$ mutant larvae or larvae treated with proconvulsant (pentylenetetrazol [PTZ] or ketopentenoate [EKP] chemical models) are represented by dark green bars (control [CTR]). A-C, Locomotor activity. Values show the mean ± SD from independent experiments (n = 3 in all conditions, except in the case of ho *scn1Lab*$^{-/-}$ and wt larvae, where n = 9) normalized to the data represented by the dark green bars. In each experiment, the individual activities of larvae (n = 10 per experiment) were pooled and processed (represented by dots). A, Homozygous *scn1Lab*$^{-/-}$ mutants. B, C, Chemical models (PTZ and EKP). D-F, Brain activity. Values show the mean ± SD of frequency of epileptiform events (n per 10 minutes) recorded in individual larvae (represented by dots). *scn1Lab* genetic model: VHC (wt) and RIZA (ho), n = 9; LIS (ho), n = 13; EFA (ho), n = 14; VHC (ho, CTR), n = 15; PTZ model: VHC and RIZA+PTZ, n = 7; EFA+PTZ, n = 9; LIS+PTZ, n = 10; PTZ (CTR), n = 21; EKP model: for each condition, n = 10-11. G, Representative examples of bioluminescence recordings (35 minutes) of *Tg(elavl3:GA)* zebrafish exposed to VHC, PTZ, or EKP. The total photon emission (y-axis) is counted per 5-second interval (x-axis). H, I, Bioluminescence. Values show the mean ± SD of percentage change in bioluminescence relative to the one observed for *Tg(elavl3:GA)* zebrafish exposed to PTZ or EKP (black dots, CTR). In each experiment, three larvae were used simultaneously (n = 3). Number of experiments performed (results represented by dots), in the case of PTZ: VHC, n = 3; EFA+PTZ, n = 5; RIZA+PTZ, n = 6; PTZ (CTR), n = 8; LIS+PTZ, n = 9; in the case of EKP: RIZA+EKP, n = 3; VHC, n = 4; EFA+EKP/LIS+EKP, n = 6; EKP (CTR), n = 9. Statistically significant differences between the mean represented by the dark green bars (black dots) and the mean of the other conditions: *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs controls. Lack of statistically significant difference is left blank [Color figure can be viewed at wileyonlinelibrary.com]

brain activity, only EFA showed inhibitory activity but not LIS or RIZA (Figure 1E and 1F).

Bioluminescence assays revealed the excitatory neuroactivity induced by PTZ and EKP (Figure 1H). This activity was more pronounced with EKP, in line with the higher frequency of epileptiform events observed in brain recordings (Figure 1F compared to 1E). In this assay, only EFA, but not LIS or RIZA, significantly decreased the amount of

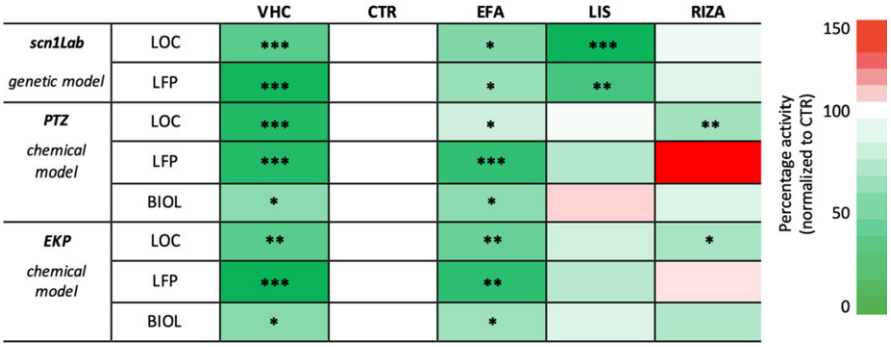

FIGURE 2 Heat map comparison of zebrafish larval responses after rizatriptan (RIZA), efavirenz (EFA), and lisuride (LIS) treatment, as examined by locomotor tracking (LOC), electrographic activity recording (LFP), and bioluminescence measurements (BIOL). Data were normalized to vehicle control (VHC)-treated mutant larvae (*scn1Lab* genetic model) or larvae treated with a proconvulsant (pentylenetetrazol [PTZ] or ketopentenoate [EKP], two chemical models; second column, 100% control [CTR]). VHC refers to VHC-treated wild-type larvae. The statistical differences mentioned in Figure 1 for the different conditions are shown: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ vs controls [Color figure can be viewed at wileyonlinelibrary.com]

PTZ- and EKP-triggered neuroluminescent activity (Figure 1H and 1I), in agreement with the results obtained using LFP recordings.

Intriguingly, fenfluramine, which was shown to significantly reduce seizure frequency in DS patients, demonstrated a somewhat similar activity profile in the PTZ and EKP models (readout: both locomotor activity and bioluminescence) as observed for LIS (see Figure S2).

To further interpret the results, a heat map was generated that provides a visual summary of the effect of the compounds on the epileptiform activities in the zebrafish models of seizures/epilepsy (*scn1Lab*$^{-/-}$ mutants, PTZ and EKP models) as evaluated by the three methods, that is, locomotor behavior, LFP brain recordings, and bioluminescence (Figure 2). Total number of movements, epileptic events, and photons emitted were normalized to the *scn1Lab*$^{-/-}$ mutants (genetic model), and the PTZ-treated and EKP-treated groups (chemical models), respectively. Overall, EFA was active in all models, whereas LIS was selectively active in the genetic zebrafish model of DS. In general, a poor response was observed to RIZA.

4 | DISCUSSION

With a view to repurpose marketed drugs for the treatment of DS, we sought to interrogate compounds using the zebrafish *scn1Lab*$^{-/-}$ mutant platform.[5] We previously demonstrated that the modulation of some 5-HT subtype receptors (ie, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_{1D}) can effectively reduce seizures in the *scn1Lab*$^{-/-}$ mutant model.[4,14] Here, we test three marketed compounds (ie RIZA,[7] LIS,[8] and EFA[9]) that are known to have a clear on- or off-target 5-HT profile.

LIS is a potent nonhallucinogenic 5-HT_{2A} agonist with no effect at the 5-HT_{2C} subtype receptor. As an antiparkinson drug, it is also endowed with dopaminergic activity, that is, by binding to D2, D3, and D4 receptors.[15] The off-target pharmacological spectrum of EFA is miscellaneous, as the compound has strong affinity for both 5-HT_{2A} and 5-HT_{2C} receptors, besides exhibiting γ-aminobutyric acid $(GABA)_A$-potentiating effects and interactions with dopamine, 5-HT, and vesicular amine transporters.[9] Triptans like RIZA, conversely, are known to exert their antimigraine therapeutic effect by means of a double 5-$HT_{1B/1D}$ agonism at receptors that are present in cerebral blood vessels and the central nervous system (CNS).[7]

To examine further their potential broader antiseizure profile, we also tested the compounds in a PTZ- and EKP-convulsant zebrafish model. EKP inhibits glutamic acid decarboxylase (GAD) that converts glutamate into GABA. Clinical evidence shows that lowered GAD activity is associated with several forms of treatment-resistant epilepsy, and the model is considered to be relevant as a platform for novel antiepileptic drug discovery.[13] Conversely, the PTZ model, which conceptually builds on $GABA_A$ inhibition, is most sensitive to GABAergic AEDs (eg, barbiturates or benzodiazepines).[12]

In general, the data showed good concordance between the three measurements. However, in some cases a divergence was observed between the locomotor and the LFP/neuroluminescence outcome, that is, in the case of RIZA (PTZ and EKP models), likely due to different concluding effects of the compounds on motor neurons, as compared to other neuronal populations.

Of all compounds, LIS had the most pronounced inhibitory effect on the epileptiform activity displayed by the homozygous *scn1Lab*$^{-/-}$ mutants but was inactive in the

chemical seizure models, in agreement with the results observed for fenfluramine.

As 5-HT_{2A} agonism is effective in treating the epileptiform events in the genetic DS zebrafish model,[4,14] it is speculated that the 5-HT fingerprint of LIS is critical to the activity seen in this study. However, it is tempting to think that the dopaminergic activity of the compound also contributed to the antiseizure effects observed. For instance, D3 agonism of LIS has been associated with potent neuroprotective effects, and also the activation of D2 class dopamine receptors are known to inhibit excitatory transmission mediated by the *N*-methyl-D-aspartate receptor.[16]

EFA was also highly active in the EKP model, as clearly assessed by all three readouts, and proved to possess a broader antiseizure profile, probably by virtue of its pharmacological polytarget signature. For instance, EFA also acts as a positive allosteric modulator of the $GABA_A$ receptor,[9] and probably this activity accounts for its inhibitory efficacy in the PTZ model. Moreover, modulation of this receptor (eg, by stiripentol) has proven to be effective in the treatment of DS patients.[2]

RIZA was mostly inactive or only marginally active. The latter outcome is somewhat surprising, as a nontriptan selective 5-HT_{1D} agonist clearly reduced seizures in the genetic DS zebrafish model, and similar activity elicited PTZ-related anticonvulsant activity in mice and inhibition of electroshock-induced seizure spread in rats.[16] Nonetheless, our zebrafish data are in line with clinical antiepileptic (side) activity not having been described for triptans, at least to our knowledge, although they are widely used in the management of migraine. Very recent data, however, might shed some light on this issue, as sumatriptan used at a low dose (1 mg/kg) exhibited anticonvulsant activity in a PTZ mouse model, whereas at a higher dose (20 mg/kg) a proconvulsant effect was seen.[17] Of interest, the latter activity was independent of 5-$HT_{1B/D}$ receptors but mediated by the inducible nitric oxide synthase/nitric oxide pathway, highlighting the complex biological response of the CNS to sumatriptan, and possibly to the triptan scaffold in general. These first interesting observations in mice could be further elaborated in the future by using the zebrafish *scn1Lab*$^{-/-}$ mutant platform, which offers the clear advantage of testing multiple combinations of triptans and receptor (ant)agonists in a straightforward way.

Taken together, these preclinical results using marketed drugs provide new avenues in the field of AED discovery and warrant further investigations, including clinical exploratory studies. We anticipate that LIS is a potential candidate for the treatment of DS. As the compound is very potent, it can also be applied transdermally,[15] which might offer some practical advantages in the treatment of young children.

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CONFLICTS OF INTEREST

This project was carried out with the support of Zogenix. L.L. and P.d.W. receive consultancy honoraria from Zogenix. Part of the data presented in this article are related to patent applications assigned to Zogenix. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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