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Brief Communication

Long-term negative impact of an inappropriate first anti-epileptic medication on the efficacy of a second anti-epileptic medication in mice

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ABSTRACT

Childhood absence epilepsy (CAE) is one of the most frequent epilepsies in infancy. The first-line recommended therapy for CAE is based on the prescription of the narrow spectrum ethosuximide and the broad spectrum valproic acid which have similar efficacy in the first 12 months. Nevertheless, some anti-epileptic drugs (AEDs) may worsen seizure duration and type in this syndrome. In line with this, we have been faced with a case of identical twins with CAE and early exposure to different anti-seizure drugs leading to divergent outcomes. From this, we hypothesized that the first AED to treat CAE may determine the long-term prognosis, especially in the developing brain, and that some situations leading to drug-resistance may be explained by an inappropriate first AED. Therefore, we investigated this hypothesis by using a genetic mouse model of absence epilepsy (BS/Orl). Mice received a first appropriate or inappropriate AED followed by the same appropriate AED. Our data demonstrate that an inappropriate first AED has a negative impact on the long-term efficacy of a second appropriate AED. This work supports the necessity to effectively diagnose epileptic syndromes prior to medication use, particularly in children, in order to prevent the deleterious effects of an inappropriate initial AED.

Keywords: absence epilepsy, long-term prognosis, mouse model, anti-epileptic drug, valproate

Short Summary

This study investigated whether a first inappropriate anti-epileptic medication (AED) would have a long-term impact on the efficacy of a subsequent appropriate AED; thus affecting the long-term prognosis. To do this, we used a genetic mouse model of absence epilepsy (BS/Orl) where mice received a first appropriate or inappropriate AED followed by the same appropriate AED. We found that an inappropriate first AED has a negative impact on the long-term efficacy of a second appropriate AED. This supports the necessity to effectively diagnose epileptic syndromes prior to medication use.

INTRODUCTION

Childhood absence epilepsy (CAE) has a prevalence of 7 in every 10000 children and is one of the most frequent epilepsies in infancy¹. Classically this epilepsy begins between 4 and 10 years of age with many absence seizures (AS) a day, as defined as an abrupt behavior arrest with a loss of consciousness concomitant to ictal EEG discharges of 3Hz generalized spike and waves, bilateral and symmetric discharges (SWD)². The first-line recommended therapy is based on the prescription of the narrow spectrum ethosuximide (ESM) and the broad spectrum valproic acid (VPA) which have similar efficacy in the first 12 months with 45% freedom-from-failure rate, including intolerable adverse effects and lack of seizure control, in particular³. Nevertheless, some anti-epileptic drugs (AEDs) such as carbamazepine (CBZ) and vigabatrin (VGB) may worsen seizure duration and clinical type in this syndrome (i.e. generalized tonic-clonic seizures can occur) and could alter the prognosis⁴⁻⁶. Fortuitously, we have been faced with a case of identical twins with CAE and early exposure to different anti-seizure drugs leading to divergent outcomes. GB started with the first absence seizures (AS) as aged 6 (1986). At that time, they presented with unremarkable early milestone development, and no family epileptic history. GB received VPA prescribed by his general practitioner as the first treatment. VPA reduced AS frequency without suppressing them. Until adulthood, he presented short lasting loss of contact (a few seconds), without myoclonic movements or automatism, when exposed to sleep deprivation or fatigue and anxiety. Despite persisting AS, VPA remained the sole antiepileptic drug regimen GB received, and he never presented with myoclonic seizures, tonic seizures or generalized tonic-clonic seizures. He never complained of falls during AS, and he managed two employments in parallel. One year later, in 1987, GB's identical twin brother, JB, presented also with AS. He was referred to a regional academic hospital where CBZ was introduced. Rather unexpectedly, AS were not reported by the parents until age 15, in contrast to his brother. However, since age 15, he presented generalized tonic-clonic seizures, then AS resumed, with myoclonic seizure, tonic and atonic seizures, with severe drug-resistance. In the following years, including adulthood, he suffered many seizures, with repeated facial trauma from falls to the ground and multiple yearly admission to emergency units. He failed in managing any type of professional activity and even leisure activities were not sustained due to frequent occurrence of severe seizures. Historically he received, alone or in association, over the years, VPA, ethosuximide, phenytoin, phenobarbital, clonazepam, VGB, lamotrigine, rufinamide, gabapentin, zonisamide, topiramate, felbamate, perampanel, clobazam, without any significant improvement. Thirty years after onset, JB still presents with monthly traumatic

falls, atypical absence with incomplete alteration of consciousness, and several general tonic-clonic seizures each year. Social activities are very poor. He is now receiving felbamate and VPA. For EEG recordings from JB and GB at 30 years of age see Figure S1.

Because these twins were initially treated with different AEDs, we hypothesized that the diverging outcomes are the result of a first unsuccessful AED which determines the efficacy of subsequent AEDs. Overall, this example sheds light on the hypothesis that we address, suggesting that a first medication to treat epilepsy could have an effect on the long-term prognosis, especially in developing brain. Hence, a poor first treatment can be responsible for a deleterious long-term outcome. To test this hypothesis we used a validated mouse model of absence epilepsy (BS/Orl) where outcomes were compared in mice receiving a different appropriate or inappropriate initial AED for absence epilepsy followed by a common, appropriate AED for this form of epilepsy. Here we use *appropriate* to refer to generally effective and commonly used medication for CAE.

METHODS

To investigate the long-term impact of a first AED treatment on the evolution of seizures after subsequent AEDs, we studied 4 groups of male BS/ORL mice, a genetic model for absence epilepsy presenting spontaneous SWD⁷ which received various initial AED treatments followed by a common AED. The care and experimental manipulation of the animals were carried out in accordance with the guidelines of the European Union (directive 2010/63/EU). At 25±2 days of age, male BS/Orl mice were implanted under general anesthesia (chloral hydrate, 400 mg/kg, i.p.) with 4 monopolar tungsten rod electrodes placed bilaterally over the frontal and parietal cortex and 1 electrode placed over the cerebellum as a reference electrode. All electrodes were connected to a female micro-connector and fixed to the skull with cyanolate and acrylic cement. One week after implantation, and every 2 weeks after the start of treatment, freely moving mice were recorded for 3 consecutive days (3 days x 1h EEG recording) for basal epileptic activities using a digital acquisition computer-based system (Coherence, Deltamed, France, max. 32 acquisition channels - sampling rate 256 Hz) in freely moving animals placed in a Plexiglas cage located in a Faraday cage. Immediately after the first (this initial) EEG recording, mice were treated daily for two weeks with either VPA (200mg/kg/day; Aguetant France, batch number 4202475), ESM (200mg/kg/day; Sigma-Aldrich France, batch number 129K1342) or VGB (500mg/kg/day; Sanofi-Aventis France, batch number 7544) dissolved in saline solution (0.9%). These doses were chosen based on previous research and what is typically used in seizure models and

experimental epilepsy in rats as well as in mice⁸⁻¹⁰. Serum drug levels were not assessed in the present study. All medications were administered daily via an intraperitoneal injection as daily medication use is typical in the clinical situation and also in animal models of absence epilepsy⁸⁻¹⁰. Thirty minutes prior to EEG measurements, AED administrations were done. After 2 weeks, EEG recordings were made to evaluate the epileptic activity after the first AED treatment, then all mice were treated only with VPA (200mg/kg/day) for 6 weeks. There was no washout period between drug administrations. EEG recordings were done every 2 weeks. An additional group of male mice only received vehicle (saline solution: PHY). This resulted in 4 groups: 1) PHY-PHY (n=5), 2) VPA-VPA (n=5), 3) ESM-VPA (n=5) and 4) VGB-VPA (n=4). See Figure 1.

For all statistical analyses, we used the cumulative duration of SWD activities (CDS), excluding the first 20-min recording which was considered habituation. At baseline, the CDS of the 4 groups of mice were compared using an ANOVA. Then, the variations in the CDS along the second AED treatment were measured for each EEG using the Response Ratio (RRatio) defined as $RRatio = \frac{T_{EEG_x} - T_{EEG_1}}{T_{EEG_x} + T_{EEG_1}} * 100$ where T_{EEG_1} is the CDS at baseline and T_{EEG_x} at EEG_x . R Ratios vary from -100 to 100, -33 corresponding a 50% reduction of the cumulative duration of seizure. At EEG_2 , ANOVAs of the R Ratios were used to assess the effect of any treatment versus saline and the differential effect of the three first treatments. Finally, a mixed effect ANOVA for repeated measures of R Ratios at EEG_2 to EEG_5 was used. We also compared trajectories using post-hoc unilateral or bilateral t-tests, respectively depending whether the superiority of one treatment to another to reduce the evolution of the cumulative duration of seizures may be hypothesized or not. Statistical significance was set at $p < 0.05$.

RESULTS

Prior to treatment EEG (EEG_1) activity did not differ between groups ($F=2.49$, $p=0.10$). After 2 weeks of treatment (EEG_2), there was a significant effect of treatment (AEDs vs PHY; $F=10.2$, $p=0.006$) and significant differences between treatments ($F=31.70$, $p < 0.0001$), with ESM treatment being the only AED to show a significant difference in response ratio at EEG_2 ($t=6.91$, $p < 0.0001$). VPA treatment presents significant higher effect than PHY treatment for reducing seizures between EEG_2 - EEG_5 ($t=1.88$, $p=0.036$). At EEG_4 - EEG_5 , the initial success of the ESM treatment did not last after VPA treatment compared to the VPA-VPA group ($t=0.51$, bilateral $p=0.63$). In addition the

negative VGB effect persisted despite a switch to VPA when comparing VGB-VPA and VPA-VPA groups ($t=3.22$, unilateral $p=0.007$) and is non-significantly different from no treatment, when comparing VGB-VPA and PHY-PHY groups ($t=1.61$, bilateral $p=0.15$). See Figure 2.

DISCUSSION

Failure of first AED to control epilepsies seems to induce (or be a predictor of) a drug-resistance especially in CAE¹¹⁻¹⁵. But as far as we know, it has never been investigated whether the outcome could be the *consequence* of the failure of the initial medication. In the introduction, we have reported the case of identical twins with CAE and early exposure to different anti-seizure drugs leading to divergent outcomes which we hypothesized may be due to different initial medications. However, it is interesting to note that the worsening of seizures in the twin who received the ‘inappropriate’ medication did not occur until age 15. We know that concordance rate in monozygotic twins is high for absence seizures^{16,17}, therefore it is very likely that both twins suffered from the same disease, presenting as CAE. According to this hypothesis, the obvious factor that led to such a divergent evolution of the disease is represented by the initial anti-seizure drug. This could suggest that some anti-seizure drugs, when initiated early in the course of the disease, could have a disease-modifying impact; as further studied in our mouse model. However, it is likely that other factors, such as the differences in the age at diagnosis, and the CAE progression, may play a role. In our mouse model, we show an inappropriate first AED may not be effective and may worsen the epileptic syndrome prognosis when this first inappropriate medication is replaced by an appropriate AED, underlying key effects on brain development. Precisely, we have observed that 1/ initial treatment with ESM and VPA have a positive effect on SWD activity with ESM greatly improving SWD; 2/ the benefit obtained in using ESM as a first line medication compared to VPA is lost when VPA is used instead of, and after, ESM; 3/ on the contrary, VPA treatment after VGB did not improve SWD activity at all, such that VGB was as ineffective as no treatment. This study suggests that a form of “acquired drug-resistance” can be promoted in CAE by using a first inappropriate treatment. At this stage, one cannot know if the deleterious effect is due to a specific stage in brain development. However, it is clear that the stage of development remains critical as illustrated by a study realized in a CAE rat model¹⁸ in which an early treatment can definitively suppress the epileptic activity. This finding has obvious implications for clinical practice since it is well known that the rate of epilepsy misdiagnosis is high, confounding between real epileptic syndromes and non-epileptic seizures¹⁹. Also, this finding highlights the

necessity to investigate the clinical effect of new AEDs when used as a first line treatment. Our work shows that an initial medication, appropriate versus inappropriate, can dramatically impact the outcome of the epilepsy. Knowing that the form of epilepsy in consideration determines the spectrum of appropriate medications, this work strongly supports the necessity to effectively diagnose prior to use of a medication, particularly in children, either by trained physicians or in specialized ‘first seizure clinics’²⁰. ESM and VPA have comparable efficacy in the short term³ despite different long-term outcomes¹¹. ESM can also have anti-epileptogenic and disease-modifying effects^{18,21}. For these two reasons, but also because ESM has a greater effect than VPA in our study, our future work will determine effects of additional medication combinations with a focus on understanding the role that ESM has as a second-line therapy.

Figure Legends.

Figure 1. Experimental treatment timeline. One week after implantation, freely moving mice were recorded for 3 consecutive days (3 days x 1h EEG recording) for basal epileptic activities. Then, mice were treated for two weeks with either VPA, ESM or VGB. After 2 weeks, EEG recordings were made to evaluate the epileptic activity after the first AED treatment, then all mice were treated only with VPA for 6 weeks. EEG recordings were done every 2 weeks. An additional group only received saline solution (PHY). Finally, this resulted in 4 groups: 1) PHY-PHY, 2) VPA-VPA, 3) ESM-VPA and 4) VGB-VPA. AED=antiepileptic drug, VPA=valproate, ESM=ethosuximide, VGB=vigabatrin, PHY=saline.

Figure 2. Impact of first treatment AED on the evolution (%) of the cumulated SWD duration normalized by natural evolution of the epilepsy (in PHY-PHY group), in four groups of BS/Orl epileptic mice. AED=antiepileptic drug, SWD=spike and wave discharges, PHY=saline.

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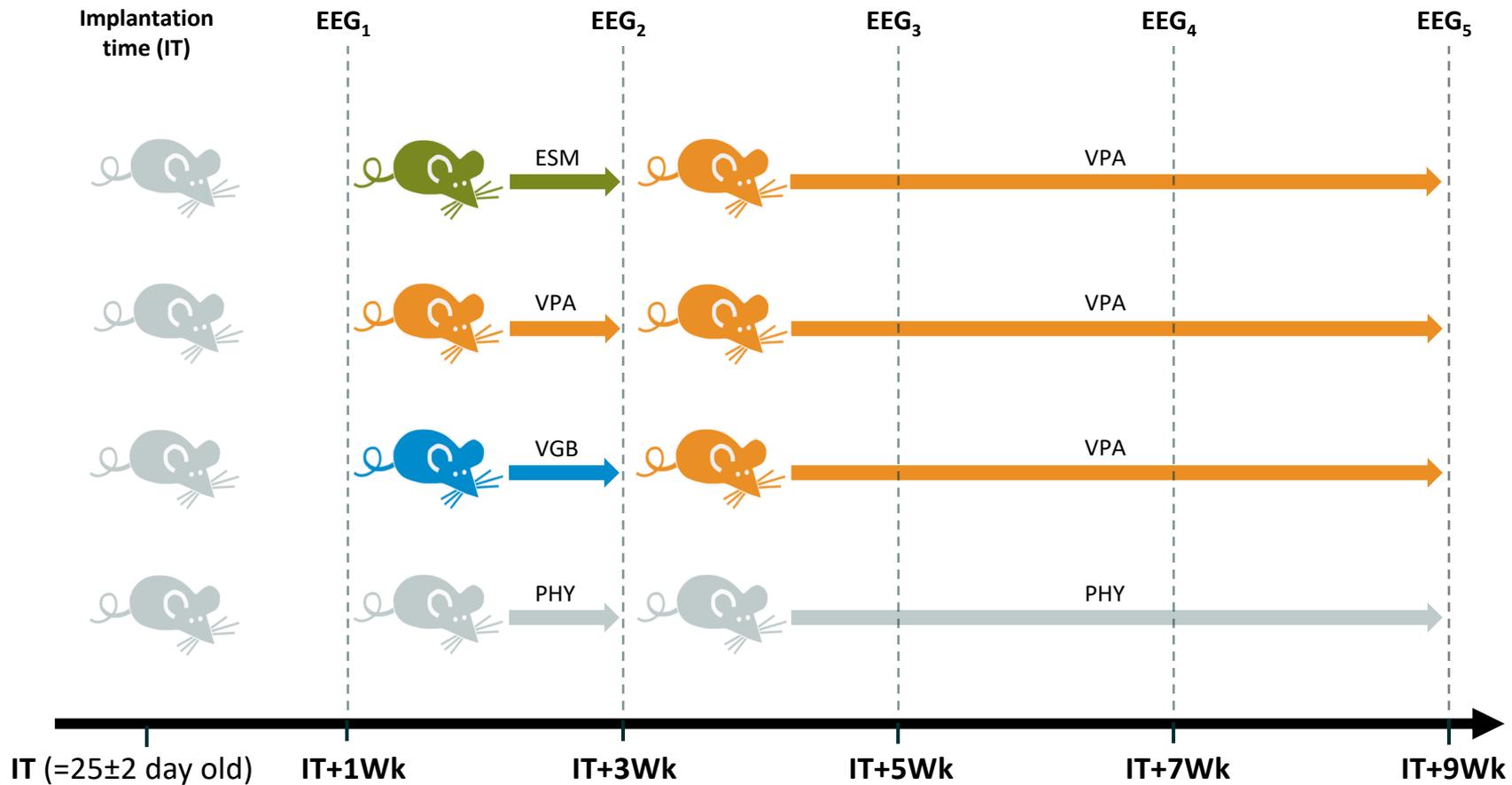
DISCLOSURE OF CONFLICTS OF INTEREST: MK, SH, GD, NC have no conflicts of interest. JLP has received an honorarium from BINC-Geneva as a speaker. LV received honorarium from UCB-Pharma and Eisai as speaker. AB received subventions from Eisai, Shire, Novartis and honorarium from Novartis, UCB-Pharma, Zogenix as member of a board. BM received subventions from Eisai and UCB-Pharma.

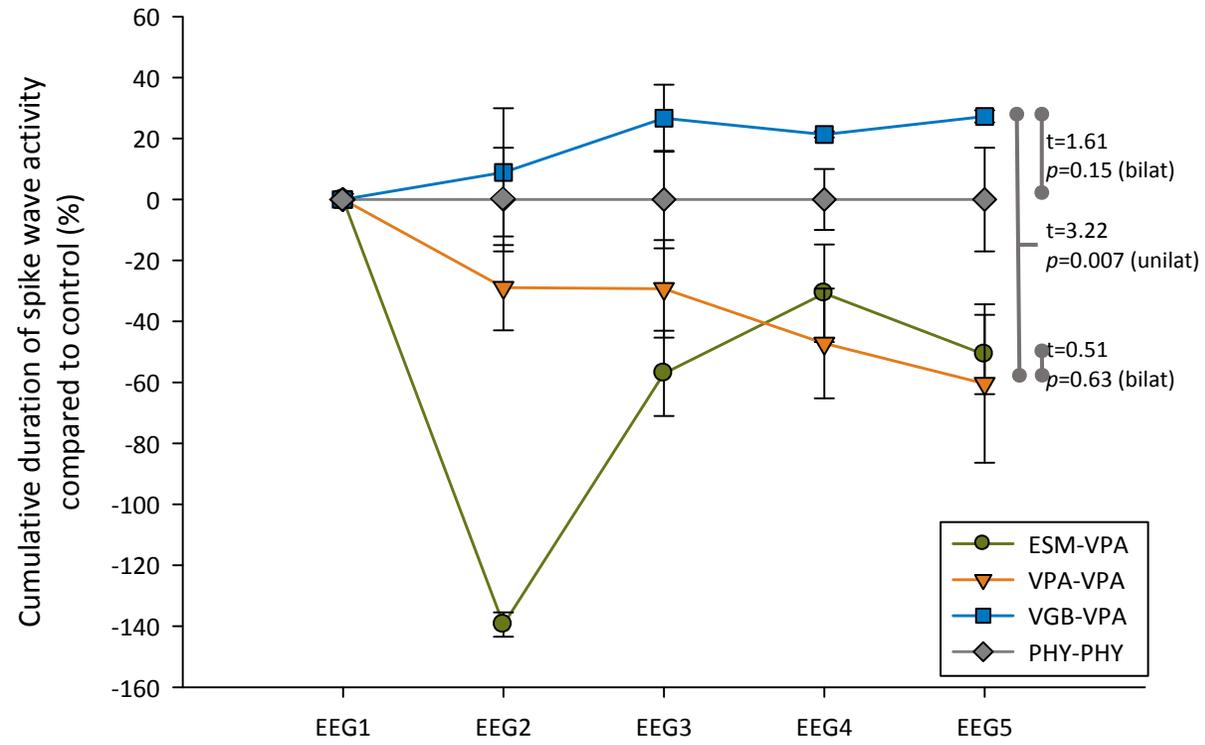
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Supplementary Figure S1.

- a. EEG traces from JB at age 30, showing: Diffuse rhythmic spike-and-wave discharge at 3Hz during 7 seconds, with only subtle change in contact (the technician wrote “RAS, M’ENTEND”, meaning “Nothing to note, (he) can hear me”). Time constant 0.3 sec, low-pass filter 120hz.
- b. Same recording from JB at age 30, with decreased awareness, causing first a burst of spike-and-wave discharge, followed by a sudden rapid low- then high-voltage diffuse polyspike activity lasting 19 seconds, with a progressive loss of posture, first left unnoticed (“RAS”), then causing the patient to fall. Time constant 0.3 sec, low-pass filter 120hz.
- c. EEG traces from GB at the same age, showing less diffuse rhythmic spike-and-wave discharge at 3-4Hz, without any change in contact. Time constant 0.3 sec, low-pass filter 120hz.

