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Certain secondary antiepileptic drugs can rescue hippocampal injury following a critical growth period despite poor anticonvulsant activity and cognitive deficits

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ABSTRACT

Clinical and experimental studies have shown that many common secondary antiepileptic drugs (AEDs) are ineffective at blocking seizures in adulthood; however, some afford neuroprotection. In early development, certain AEDs cause apoptosis; however, it is unknown whether these drugs are neurotoxic to the juvenile brain following a developmentally regulated proapoptotic period and whether they alter the seizure threshold, seizure-induced neuronal vulnerability, and/or cognitive function. Lamotrigine (LTG), carbamazepine (CBZ), phenytoin (PHT), valproate (VPA), and topiramate (TPM) were systemically administered to rat pups for 7 days beginning on postnatal (P) day 14 (P14), then half the animals were injected with kainate (KA) to trigger seizures, an age when the CA1 subregion becomes preferentially sensitive to status epilepticus. Histological outcome, seizure severity, and learning and memory were determined with an electroencephalograph (EEG), silver impregnation, and a water-maze swim task. None of the AEDs tested significantly attenuated behavioral or electrographic seizures. Phenytoin increased mortality, identifying a detrimental side effect of this drug. The other drugs (LTG, VPA, TPM, and CBZ) afforded different amounts of protection to the CA1 subregion but not to the CA3 subregion or extrahippocampal structures. With the exception of VPA, AED-treated animals lagged behind during swim task acquisition. All groups improved in the water-maze swim task over time, particularly on the last trials; however, the average escape latency was still impaired for TPM-treated animals and all AED + KA-treated groups. Thus, while certain AEDs demonstrated some neuroprotective effects, poor antiepileptic activity, memory impairment, and other deleterious side effects were observed with these drugs suggesting that the search for potentially more effective and tolerated agents is essential for improving clinical outcome in children and adolescents with epilepsy.

1. Introduction

Children are at a particularly high risk for seizures which can occur in association with cerebral palsy, mental retardation, epilepsy, and general cognitive impairment, as well as a high mortality rate following neonatal seizures [1–7]. In addition, certain types of epilepsy that begin early in life are often of a different pathology than those that begin in adulthood [8]. For example, clinical and experimental evidence reveals that status epilepticus triggers a cascade of events in the immature brain that lead to acute epileptic encephalopathy and autoimmune phenomena which may eventually cause neuronal loss and learning deficits [9–16]. Despite the high rate of seizures in infants and children and the demonstration of deficits and fatality that they cause, safe and effective treatment for early-life seizures, particularly during febrile sensitive years, is still needed. In experimental epilepsy, phenobarbital (PB) and commonly used secondary AEDs such as valproate (VPA) or phenytoin (PHT), when administered during the brain "growth spurt" (P0–P14), can induce neuronal apoptosis, the highest level being at P7 [17–21]. In humans, this period begins in the 3rd trimester of gestation and ends by the third year of life [22]. Newer AEDs such as lamotrigine (LTG) (3,5-diamino-6-2,3-dichlorophenyl-1,2,4-triazine), also used to treat various types of clinical seizures [23,24], were reported to reduce the seizure threshold in adulthood when rats were exposed during the second postnatal week (P7–P14) [20]. Lamotrigine and phenytoin, having similar anticonvulsant profiles that can block L-type Ca^{2+} and voltage-gated Na^{+} channels in a use-dependent manner, can cause a decline in glutamate release [25–28].

Currently, many available antiepileptic drugs (AEDs) used in the adult population have deleterious side effects such as depression, cognitive impairment, or toxicity as well as susceptibility to clinically important drug–drug interactions [18,19,22,29]. In adult animals, experimental seizure studies showed that treatment with LTG neither attenuates synchronous activity in the electroencephalograph (EEG) nor prevents the associated impairment of cognitive function in the Morris water-maze swim task [30,31]. Despite these null findings, in adult seizure models, LTG has been found to have paradoxical neuroprotective effects whether it is

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administered before or just after the epileptic episode [30,31]. Moreover, in mature animals, controversial results have also been reported for carbamazepine (CBZ), also a Na^+ ion channel blocker. Carbamazepine was reported to reduce epileptic activity [25]. However, recent investigation also suggests that it does not attenuate status epilepticus-induced cognitive impairment, reduce many phases of epileptic activity, nor protect from injury [32]. In fact, direct application of CBZ to cultured cerebellar neurons can be toxic [33], and high doses in vivo (100 mg/kg) can also induce cell death [29]. Although neuroprotective effects have been well demonstrated in both in vitro and in vivo models for PHT, neurodegeneration has also been observed when it is applied to cerebellar cultures [32,34]. Valproate (VPA), a sodium and T-type calcium channel blocker and enhancer of GABAergic transmission [20,35] also clinically used to treat a wide range of seizure types, has a number of deleterious side effects [36]. For example, when VPA is administered chronically, nonspecific neuronal lesions were observed [37]. Unlike PHT and VPA, topiramate (TPM), a newer secondary AED proposed for the treatment of early-life seizures without neurotoxic "side effects" at clinical doses [18,38,39], was also found to cause a significant amount of apoptosis when administered during the first postnatal week [39].

In contrast, there is a dearth of research on the effects of these secondary AEDs on neurotoxicity, cognition, and the seizure threshold just prior to and during the onset of the juvenile period, when seizure-induced CA1 neuronal injury is first prominent that coincides with a surge in glucocorticosteroids [40,41]. Since 7 days of treatment was previously tested during the first two weeks of rat development, which approximates 7.5–12 months of human development [20], we exposed rat pups during the 2nd to 3rd postnatal weeks of rat development. This reflects an age group of approximately 2–5 years, the most common time when febrile seizures occur in childhood prior to puberty [42]. Since the efficacy on seizure threshold and the potential deleterious side effects of these common secondary AEDs in young children are unknown, the present study examined the effects of five common secondary AEDs (LTG, CBZ, PHT, VPA, and TPM) on growth, mortality, seizure severity, neurotoxicity, and learning and memory near the end of the growth spurt and proapoptotic period [15-17,43]. The doses used for all of the AEDs tested fell in the dose range previously examined by others that does not induce apoptosis [19,20,45]. Poor antiepileptic activity and other deleterious side effects were observed during the juvenile period, further emphasizing that new drugs to aid epilepsy in children and adolescents need further exploration.

2. Materials and methods

2.1. Animals

Male and female Sprague–Dawley rats (P14–P24) (Charles River, St. Louis, MO) were housed in single cages with their lactating mother until sacrifice and given food and water ad libitum. Animals were kept on a 12-h light/dark cycle at room temperature (55% humidity) in our own accredited animal facility. All animal procedures were in accordance with NIH guidelines. Animals were divided into control (N = 14) and experimental groups (N = 80).

2.2. Drugs

Each of the five drugs (lamotrigine: LTG, 10 or 20 mg/kg; carbamazepine: CBZ, 20 mg/kg; phenytoin: PHT, 20 mg/kg; valproate: VPA, 50 mg/kg; topiramate: TPM, 50 mg/kg) or vehicle (dimethyl sulfoxide: DMSO, 5%) was administered (i.p.) daily for 7 days to rat pups beginning on P14. The drugs LTG, CBZ, PHT, and TPM were first dissolved in DMSO. The final concentration of DMSO was ≤5% for any of the drugs used. For example, 40-mg LTG and CBZ stocks were dissolved in DMSO (100%), then diluted, 1 part drug with 19 parts dH_2O (20 mg/kg final); LTG was further diluted by 50% to also test nonapoptotic doses (10 mg/kg, N = 5). For PHT, 40-mg PHT was dissolved in 100% DMSO and then diluted, 1 part drug with 39 parts dH_2O. For TPM, the stock (100 mg) was dissolved in 50% DMSO, then diluted, 1 part drug with 19 parts dH_2O, and followed by sonication for 10 min. Valproate was readily dissolved in distilled water for intraperitoneal administration. Antiepileptic drug treatment was also continued daily after the KA-induced episode until the rats were perfused for tissue analysis on P24. KA was dissolved in phosphate buffered saline (PBS) by vigorous vortexing and addition of several drops of 3N NaOH to a stock concentration of 12.5 mg/ml, pH 7.4.

2.3. Induction of status epilepticus

To induce status epilepticus, the animals received a single injection of kainate (KA) (7 mg/kg, i.p.) on P21 1 h following the last treatment of secondary AEDs. Behavioral seizure scoring was performed for 2 h after seizure onset as previously described [44]. In order to avoid additional stress (separation from the mother) following the seizure recording observation period, juvenile control (n = 14), KA-treated (KA, n = 12), and AED-treated experimental animals (numbers are given in Tables 1 and 2) were returned to their lactating mother until sacrifice at 72 h.

2.4. Behavioral analysis of seizure severity

Behavioral motor seizure activity was classified and ranked for 2–3 h according to our modified Racine's scale [44]. The number, frequency, and duration of different behavior manifestations were scored every 5 min for 2 h, tabulated, and assessed by three experimenters. Each score was defined as follows: score 0: no detectable seizure activity; score 1: scratching; score 2: falling, head nodding; score 3: rearing; score 4: circling and running; score 5: jerking and tonic behavior; and score 6: tonic–clonic behavior, convulsions, and jumping. Behavioral observations lasted up to 4 h after KA administration. After the seizure recording observation period, juvenile control and experimental animals were returned to their lactating mother for 48 h, then tested in the memory task on P23 and P24 in the morning followed by sacrifice by perfusion on P24 in the late afternoon. Behavior-rated seizure scores in the presence and absence of AED treatments were averaged and subjected to statistics.

2.5. Electrode implantation for EEG recordings

To obtain EEG recordings, control and experimental rats were anesthetized with a mixture of 70-mg/kg ketamine and 6-mg/kg xylazine and stereotaxically implanted with bipolar electrodes in the right hippocampus on P20 at the end of the AED treatment as described (coordinates in mm with respect to bregma: AP: −3.2; L: 2.6; D: −2.8; incisor bar: −3.5) [44–46]. The electrodes were perpendicular, angled at 0° from the vertical plane. After surgery, dental acrylic was used to close the wound and hold the electrode assembly in place. The rats recovered from anesthesia and became active 1–2 h following the surgery. Animals were kept warm at 30 °C in a clean cage

Table 1
Seizure and mortality ratings.

Treatment	Seizure score	Mortality rate	N
KA	3.12 ± 0.44	14%	14
LTG + KA	3.02 ± 0.35	31%	13
CBZ + KA	4.38 ± 0.66[a]	17%	6
PHT + KA	3.80 ± 1.02	44%[*]	9
VPA + KA	3.75 ± 0.69	25%	8
TPM + KA	2.93 ± 0.4677	20%	5

There were no significant relationships between any pair of variables; Spearman correlation coefficient = −0.058, $p < 0.05$. There was a tendency towards a proconvulsant effect with CBZ pretreatment.

[a] $p = 0.08$.

[*] One way-ANOVA, $p < 0.05$.