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Effects of in utero exposure to valproate or levetiracetam on the seizures and newborn histopathology of genetic absence epilepsy rats

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ABSTRACT

Valproate (VPA) and levetiracetam (LEV), the two broad spectrum antiseizure drugs with antiabsence effects were previously tested for their antiepileptogenic effects when administered in the early postnatal period and revealed possible modification of the epileptogenic process though the effect being not persistent. The aim of this study was to investigate the effects of in utero exposure to these drugs on the absence epilepsy seizures of Genetic Absence Epilepsy Rats from Strasbourg (GAERS) rats on electroencephalogram (EEG) which are characterised by bilateral, symmetrical, and synchronized spike-and-wave discharges (SWDs). Considering LEV was proposed as a safer drug of choice in pregnancy, its effects on the newborn histopathology of GAERS was also investigated. Adult female GAERS were randomly grouped as VPA-(400 mg/kg/day), LEV- (100 mg/kg/day), and saline-treated. The drugs were injected into the animals intraperitoneally starting before pregnancy until parturition. The lungs, kidneys, and brains of the LEV-exposed newborns were evaluated histologically to be compared with unexposed naïve Wistar and GAERS newborns. Rest of the VPA-, LEV-, and saline-exposed offsprings were taken for EEG recordings on postnatal day 90. VPA or LEV did not show significant effect on mean cumulative duration and mean number of SWDs on EEG. The lungs of the LEV-exposed offsprings showed thickened alveolar epithelium in most regions, suggesting incomplete development of the alveoli. The renal examination revealed dilated Bowman's spaces in some renal corpuscles, which may be interpreted as a deleterious effect of LEV on the kidney. In addition, brain examination of LEV- and saline-exposed groups revealed irregularities in cortical thickness compared to Wistar control group. Lack of significant difference on SWD parameters may indicate that the mechanism responsible for the antiepileptogenic effects of VPA and LEV may not be operating in the prenatal period. The detrimental effect of LEV exposure observed in our study on the lungs and the kidneys of the newborns should be investigated by further studies with advanced molecular and biochemical techniques.

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1. Introduction

Generalized seizures of absence epilepsy, prevalent especially in children 5–15 years of age, are characterized by impaired consciousness with bilateral, symmetrical, and synchronized spike-and-wave discharges (SWD) at 2.5–4 Hz frequency on electroencephalogram (EEG) [1,2]. Genetic Absence Epilepsy Rats from Strasbourg (GAERS) is one of the well-defined and validated animal models which share similar ictal characteristics with human absence seizures and similar pharmacoresponse to the antiseizure drugs (ASD) [3].

Epileptogenesis refers to "the development as well as structural and functional extension of brain tissue capable of generating spontaneous seizures, resulting in the development of a chronic epileptic condition and/or progression of epilepsy after the condition is established" [4] and this process is suggested to be similar both in acquired and genetic forms of epilepsy [5]. Epileptogenesis of genetic absence epilepsy is associated with the maturation of cortical discharges in the somatosensory cortex (SoCx) in GAERS. SWDs, which continue throughout the lifespan of GAERS, initially appear around postnatal day (PN) 30 as immature discharges, are observed in approximately 30% of GAERS on PN40, and in all animals by the age of 3 months [3]. Valproate (VPA) and levetiracetam (LEV), the two broad spectrum ASDs with antiabsence effects [6,7], have been tested for their antiepileptogenic effects. A study in GAERS has assessed the antiepileptogenic effects of LEV from PN23 until PN60 at an intraperitoneal dose of 54 mg/kg/day [8]. Although epileptiform discharges were found to be reduced when measured on the first four days after the treatment, this effect did not persist until PN120-124 [8]. Similarly, reduction in SWDs were also observed in WAG/Rij rats – which is another validated model of absence epilepsy – under the early long-term oral treatment with VPA [9] and LEV [1,10] although persistence of this effect was not examined beyond 4–7 weeks after the treatment withdrawal. In a study in GAERS, however, treatment with VPA at an intraperitoneal dose of 200 mg/kg/day before the development of epileptiform activity, from PN5 until PN25, was found to be ineffective at altering the development of SWDs measured up to PN105 [11]. Also, in WAG/Raj rats, the early long-term oral treatment with LEV showed only a temporary antiepileptogenic effect after the treatment [12].

Of the drugs used in our study, VPA is notorious for being the most teratogenic ASD with its association to major congenital malformations such as spina bifida, atrial septal defect, and cleft lip-palate [13], whereas LEV has much less relation to these pathologies and is proposed as a safer drug of choice for pregnancy [14,15]. However, several case-reports suggest that LEV users may show evidence of drug-induced lung diseases [16–18] and nephritis [19].

We hypothesised that the in utero exposure to VPA and LEV may modulate epileptogenesis of GAERS and assessed whether intraperitoneal administration of these agents throughout the pregnancy affect SWDs in the offsprings of GAERS when the SWDs were maturated at the age of PN90-100. Additionally, following the in utero exposure to LEV throughout the pregnancy, we assessed the histopathological effects in the newborns.

2. Methods

2.1. Animals and experimental design

Female adult GAERS (n = 22) and a female adult Wistar rat were taken from the breeding colony of the Department of Medical Pharmacology of Marmara University School of Medicine. The animals were accommodated in a controlled environment with a temperature of 21 ± 3 °C and 12-hour light/dark cycle (lights on at 8 a.m.). Free access to food and water were provided to all animals.

The GAERS animals were randomly grouped as saline-treated (n = 6), VPA-treated (n = 8), and LEV-treated (n = 8). Every two female rats from the same treatment group were placed together in a mating cage with a randomly selected male GAERS, with a total of three rats per cage. Twice daily saline, VPA, or LEV treatments were started on the first day of animals in the mating cage and continued until parturition. Remaining female control GAERS (n = 1) and female control Wistar rat (n = 1) were taken to two separate mating cages and placed with a randomly selected male GAERS or Wistar rat, respectively. Total number of pups at birth and the number of pups reached into adulthood were evaluated for each group except for the randomly selected LEV-treated GAERS (n = 1), female control GAERS (n = 1), and female control Wistar rat (n = 1) since it was not possible to predict whether alive animals would survive into adulthood if were not sacrificed for the histopathological examination.

The LEV-exposed newborns (n = 8), but not the VPA-exposed newborns due to high rate of stillbirth, were examined histopathologically and were compared with unexposed naïve Wistar (n = 4) and GAERS (n = 4) newborns as control groups. The remaining animals underwent stereotaxic surgery at PN80–90 for placement of the EEG electrodes and were kept in their cages for a recovery period of one week. Following the recovery, 3-hour EEG recordings were taken from saline- (n = 8), VPA- (n = 10), or LEV-exposed (n = 11) animals.

All animals were treated according to the approval of the Ethical Committee for Experimental Animals of Marmara University (Protocol number: 108.2018.mar) and conformed to the Directive 2010/63/EU of the European Parliament and of the Council.

2.2. Drug preparation and injection procedure

The doses of VPA (200 mg/kg) and LEV (50 mg/kg) were determined to provide effective control of the seizures [6,20]. VPA (Depakin, 400 mg/4 mL) or LEV (Keppra, 500 mg/5 mL) were dissolved in 2 mL or diluted with 5 mL of saline (0.9% NaCl) for dose adjustment. Final solutions of saline, VPA (200 mg/mL), or LEV (50 mg/mL) were injected intraperitoneally at a volume of 1 mL/kg body weight according to their respective groups twice daily (at 10 a.m. and 4p.m.) starting from the first day of their placing in mating cages until parturition.

2.3. Stereotaxic implantation of EEG electrodes in the GAERS at PN80-90 that were exposed to saline, VPA, or LEV in utero

The rats were anesthetised with ketamine (100 mg/kg, IP) and xylazine (10 mg/kg, IP) and placed in a stereotaxic frame (Stoelting Model 51600, Stoelting Co., Illinois, USA). A longitudinal incision was cut over the skull and four stainless steel screws with insulated wires were implanted bilaterally over the frontoparietal cortex for cortical EEG recordings. The electrodes were connected by insulated wires to a micro connector for the EEG recordings. The electrodes and wires were covered by dental acrylic and fixed to the skull.

2.4. EEG recordings and evaluation

SWD complexes were identified if the duration was longer than 1 s, with each sharp spike followed by a slow wave (7–11 Hz) at an amplitude at least twice the background amplitude of the EEG. EEG was amplified through a BioAmp ML 136 amplifier, with an anti-aliasing filter set at 0.1–125 Hz and digitised at a sampling rate of 1000 Hz. The data were analysed using LabChart v8 software (PowerLab 8/35, ADI Instruments, Oxfordshire, UK). The recordings were analysed according to their mean cumulative duration of SWDs, mean number of SWDs, and mean duration of each SWD for 3 h.

2.5. Histological preparation and scoring of the newborns of naïve Wistar control, naïve GAERS control, and GAERS exposed to LEV in utero

After applying experimental methods, lung, kidney, and brain samples obtained from the newborn rats were immersion fixed in 10% formaldehyde then underwent routine histological assessments. Briefly,

tissues were dehydrated in ascending alcohol series, cleared in xylene, and embedded in paraffin. Haematoxylin and eosin (H&E) dye was used to stain 4 μm-thick paraffin sections for light microscopic evaluation. In each section, at least five similar areas were evaluated blindly by histologists. Modified histopathological scoring system [21,22] was summarized in Table 1. Maximum score for each organ was 3. All stained sections were evaluated through a photomicroscope (Olympus BX51, Tokyo, Japan) and photographed with a CCD camera (Olympus DP 72, Tokyo, Japan). VPA-exposed newborns were not evaluated histopathologically due to the high rate of stillbirth.

2.6. Statistical analysis

All statistical analyses were performed with GraphPad Prism version 9.1.1 (GraphPad Software, San Diego, USA). The data were assessed for normality using a Shapiro-Wilk normality test. Unpaired *t*-test was used to analyse the time period to give birth between saline-treated, VPA-treated, and LEV-treated GAERS. Total number of pups and number of pups reached into adulthood were analysed using Mann-Whitney *U* test. For comparing mean cumulative duration of SWDs, mean number of SWDs, and mean duration of each SWD between saline-exposed, VPA-exposed, and LEV-exposed GAERS, a two-way repeated measures ANOVA followed by the post-hoc Bonferroni test (two factors: "Group" and "Time") was used. For the possible impact of sex differences on the SWD parameters, Mann-Whitney *U* test was used. The histopathological scores obtained from the semi-quantitative scoring system (Table 1) for the assessment of the lung, kidney, and brain tissue of naïve Wistar control, naïve GAERS control, and LEV-exposed GAERS were analysed with Mann-Whitney *U* test and expressed as median values since the median is considered as the most appropriate measure of central tendency for ordinal data [23]. The data other than the histopathological scores were expressed as mean ± SEM. P-values of < 0.05 were considered statistically significant.

3. Results

3.1. The effects of VPA or LEV treatment on the time period to give birth and number of the pups

The time between the beginning of the treatments and parturition was found to be 23.33 ± 1.51 days for the saline-treated group (n = 6), 27.88 ± 2.58 days for the VPA-treated group (400 mg/kg/day, n = 8), and 26.00 ± 2.63 days for the LEV-treated group (100 mg/kg/day, n = 6). The time between the beginning of the VPA and parturition was statistically longer when compared to the saline-treated group ($p < 0.05$) (Fig. 1).

Total number of pups observed at birth were evaluated as 7.38 ± 1.07 for the saline-treated group, 4.25 ± 0.96 for the VPA-treated group, and 5.50 ± 1.28 for the LEV-treated group. The difference between the groups showed statistical significance only for the saline-treated and the VPA-treated groups ($p < 0.05$). The number of pups reached into adulthood was 5.88 ± 1.42 for the saline-treated group, 1.63 ± 0.96 for the VPA-treated group, and 2.86 ± 1.35 for the LEV-treated group. Similarly, the difference was statistically significant only between the saline-treated and the VPA-treated groups ($p < 0.05$).

Table 1
Criteria and scoring system used in histopathological evaluation of the animals.

Organs	Histopathological score parameters	Score
Lung		
	Completed alveolar sac formation with thin alveolar epithelium	3
	Partially completed alveolar sac formation with thick alveolar epithelium	2
	Uncompleted alveolar sac formation with thick alveolar epithelium, presence of undifferentiated cells and uncompleted lumen formation in the blood vessels	
Kidney		
	Regular cortex formation	1
	Presence of undifferentiated mesenchymal interstitial connective tissue, occasional renal corpuscle abnormalities with dilatation of Bowman space	3
	Presence of undifferentiated mesenchymal interstitial connective tissue, diffuse renal corpuscle abnormality with dilatation of Bowman space and glomerular development retardation	2
Cortex	Regular cortical layer formation	3
	Irregular cortical layer formation	2
	Irregular cortical layer formation and deep peripheral cortex invaginations, presence of undifferentiated proliferative cells beneath the meninges	1

3.2. The effects of in utero exposure to VPA or LEV on SWD parameters

EEG recordings of the offsprings, recorded at the age of PN90-100, were analysed for their mean cumulative duration of SWDs, mean number of SWDs, and mean duration of each SWD for 3 h (Fig. 2).

Analysis of saline-exposed group (n = 8) revealed mean cumulative duration of 2689.56 ± 439.03 s and mean number of 230.88 ± 13.95 with mean duration of 11.86 ± 1.95 s for each SWD recorded. VPA-exposed group (n = 10) had mean cumulative duration of 2348.50 ± 140.78 s and mean number of 229.75 ± 20.55 with mean duration of 10.58 ± 0.66 s for each SWD recorded. LEV-exposed group (n = 11) had mean cumulative duration of 2398.01 ± 191.41 s and mean number of 207.18 ± 11.90 with mean duration of 11.79 ± 0.94 s for each SWD recorded.

The data was also analysed for the possible impact of in-group sex differences on mean cumulative duration of SWDs, mean number of SWDs, and mean duration of each SWD. Saline-exposed animals were all males hence not analysed. LEV-exposed animals (7 females, 4 males) did not show significant in-group difference between sexes for none of the measured EEG parameters. VPA-exposed animals (3 females, 7 males) did not show significant in-group difference except for mean duration of each SWD recorded, which was barely significant ($p = 0.047$). The observed significance should be interpreted cautiously due to low sample size for in-group analyses and high numbers of analyses performed, all increasing the possibility for a type I error.

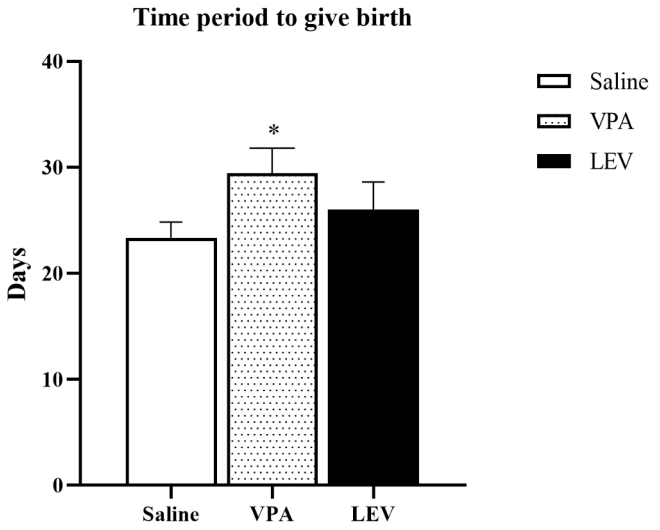

Fig. 1. The effects of VPA or LEV on time period to give birth. The time period between the first injections and birth is shown in the figure (*$p < 0.05$; Mann Whitney *U* test showed a statistically significant prolongation of the time period to give birth in the VPA group). Data were expressed as mean ± SEM. No statistical significance was found between saline and LEV groups.

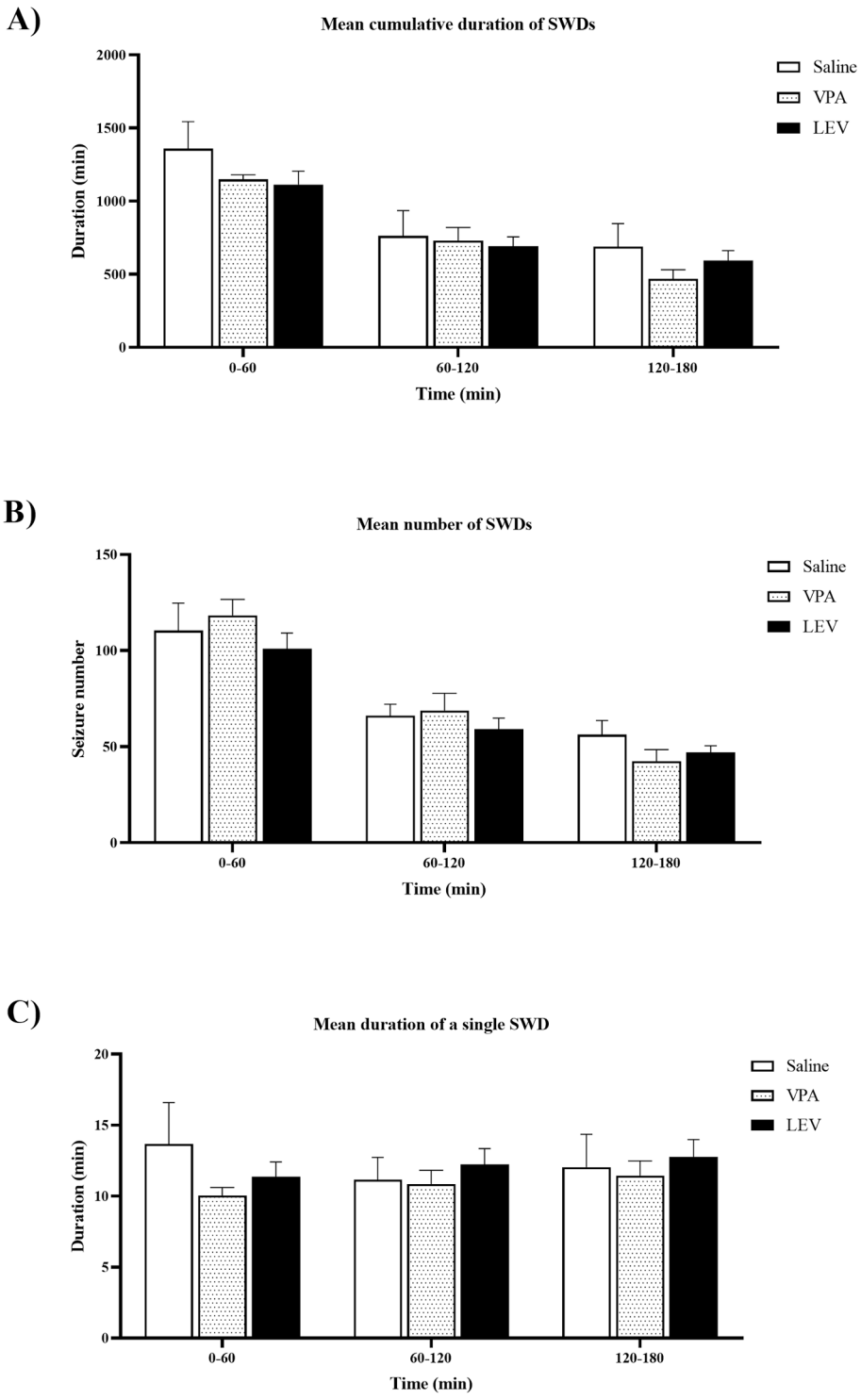

Fig. 2. The effects of the in utero exposure to VPA or LEV on cumulative duration (A), number (B), and mean duration (C) of SWDs. Data of 3-hour EEG recordings taken from the saline-, VPA-, and LEV-exposed groups are shown in 60-min epochs. Data were expressed as mean ± SEM. None of the parameters has shown statistical significance between the groups.

3.3. The effects of in utero exposure to LEV on histopathology of the newborn GAERS

The unexposed naïve Wistar newborns weighed 6.40 ± 0.27 g (n = 4) while the unexposed naïve GAERS (n = 4) and the LEV-exposed GAERS (n = 8) newborns weighed 5.27 ± 0.40 g and 5.25 ± 0.17 g, respectively. Statistical significance was found only between the unexposed naïve Wistar and the LEV-exposed GAERS ($p < 0.01$). Lack of significance between the unexposed naïve Wistar and the unexposed naïve GAERS is probably caused by inadequate sample size rather than a real effect of the treatment.

In lung evaluation of the unexposed naïve Wistar control group, alveolar sac formation with thin walls was mostly completed, but it was occasionally found to be thick. In the unexposed naïve GAERS control group, alveolar sac epithelium was thick in most regions, and with its normal morphology and thickness in some areas. On the other hand,

alveolar sac was mostly thick in the LEV-exposed newborns. Additionally, lumen formation was not completed in the blood vessels of rats in this group (Fig. 3A–C). The LEV-exposed newborns had significantly lower scores with a median of 1 ($p < 0.01$) compared to both the unexposed naïve Wistar and GAERS control groups (Table 2).

In renal evaluation, regular kidney cortex morphology with renal corpuscles and tubules was present in the unexposed naïve Wistar and GAERS control groups. Interstitial mesenchymal connective tissue was also observed in the unexposed naïve GAERS control animals. Kidney cortex of the LEV-exposed GAERS had irregularities with renal corpuscles and tubules. Additionally, there was mesenchymal connective tissue in between the nephrons and some renal corpuscles showed dilated Bowman's space in this group (Fig. 3D–F). The LEV-exposed newborns had significantly lower scores with a median of 2 ($p < 0.01$) compared to both unexposed naïve Wistar and GAERS control groups (Table 2).

In brain evaluation of the unexposed naïve Wistar control group, cortical morphology and thickness of layers were regular. However, in the unexposed naïve GAERS and LEV-exposed GAERS groups, cortical layers showed irregular thickness and abnormal proliferation at the peripheral region accompanying deep peripheral invaginations with the increase of neurons in some of the cortical areas. Additionally, in the LEV-exposed GAERS, there were undifferentiated and parallelly oriented proliferative cells beneath the meninges (Fig. 3G-I). Though the LEV-exposed newborns had lower scores with a median of 1.75, the result did not differ significantly (Table 2).

Table 2
Median scores of the histopathological scoring of the animals.

Organs	Naïve Wistar control	Naïve GAERS control	GAERS + LEV
Lung	2.50	2.25	1*
Kidney	3	3	2 *
Cortex	3	2	1.75

* $p < 0.01$ (compared with Wistar and GAERS + saline). GAERS Genetic Absence Epilepsy Rats from Strasbourg; LEV levetiracetam.

4. Discussion

4.1. The effects of VPA or LEV on time period to give birth

The treatment with VPA, but not LEV, significantly prolonged the time period to give birth. The significantly longer time period in VPA-treated group (400 mg/kg/day) may be caused both by the adverse effects of the drug on the female reproductive system and on sexual behaviour of the animals, though the latter is observed mostly in males [24] without enough evidence regarding females. Increased gestational time as a third option is unlikely considering the association of VPA use during pregnancy with preterm birth in humans [25].

The long-term use of VPA has been associated with a higher

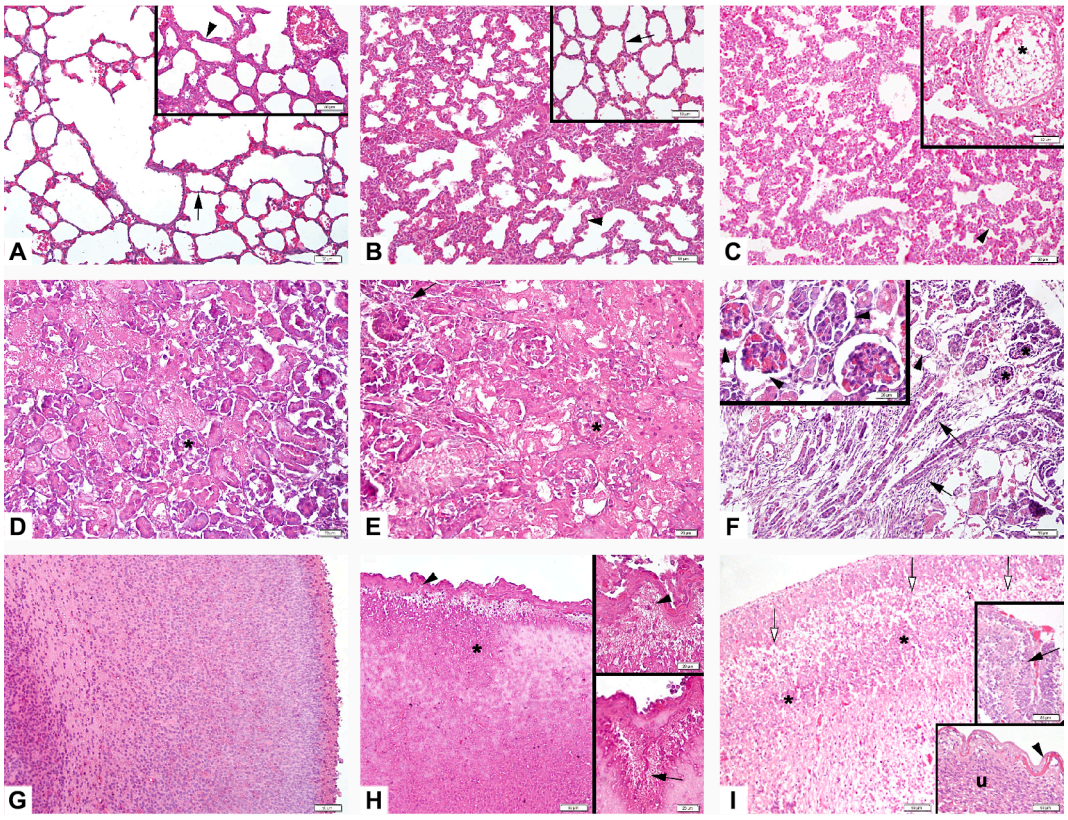

Fig. 3. Representative light micrographs of lung (A–C), kidney (D–F) and brain (G-I), samples of the experimental groups. Regular alveolar formation with thin alveolar walls (black arrow) in most regions and alveoli with thick walls (arrowhead) were seen in naïve Wistar control group (A). Alveolar sac with thick epithelium (arrowhead) in most regions and regular alveolar sac with thin epithelium (black arrow) in some areas were seen in naïve GAERS group (B). Alveolar sac with thick epithelium (arrowhead) in most regions and uncompleted blood vessel lumen formation (*) were seen in LEV-exposed GAERS group (C). Regular kidney cortex with renal corpuscles (*) and tubules were present in naïve Wistar (D) and naïve GAERS (E) control groups. Interstitial mesenchymal connective tissue (arrow) was present in naïve GAERS control group (E). Regular renal corpuscles (*) and degenerated renal corpuscles with dilated Bowman's space (arrowhead), interstitial mesenchymal connective tissue (black arrows) was present in LEV-exposed GAERS group (F). Regular cortical morphology and thickness of layers were seen in naïve Wistar control group (G). Irregular thickness of layers (*), abnormal proliferation at the peripheral region (arrowheads) and deep peripheral invagination (black arrow) with the increase of neurons were seen in naïve GAERS control group (H). Evident irregular thickness of layers (white arrows) and irregular distribution of neurons in cortical layers (*), deep invagination (black arrow) and abnormal proliferation of the peripheral region (arrowhead) with undifferentiated cells (u) beneath the meninges were seen in LEV-exposed GAERS group (I). H&E staining, scale bars: A–C and F-I 50 μm; D, E and insets in F and H: 20 μm.

incidence of polycystic ovarian syndrome (PCOS) in humans, including components of hyperandrogenism, multiple ovarian cysts, anovulatory cycles, hirsutism, and obesity. Moreover, replacing VPA with LEV is suggested to reverse VPA-associated abnormalities in hyperandrogenism, hyperinsulinemia, and low serum high-density lipoprotein cholesterol [26]. A study on adult Wistar rats showed that VPA at doses 400 mg/kg/day and 600 mg/kg/day increases the number of ovarian follicular cysts and decreases the number of corpora lutea and ovarian weight dose-dependently [27]. An *in vitro* study on effects of VPA on androgen biosynthesis in ovarian theca cells of normal-cycling women demonstrated that VPA at doses used to treat epilepsy results in increased androgen and decreased progesterone production after 72 h of treatment [28]. On the contrary, LEV had effects only on non-gonadotropin-stimulated testosterone and oestrogen secretion in porcine ovarian follicles while VPA had both on non-stimulated and stimulated secretion [29]. Changes in the reproductive system suggested in these studies may be responsible for the longer time period to give birth observed in our experiment in VPA-treated group and could explain the absence of this effect on LEV-treated group.

4.2. The effects of in utero exposure to VPA or LEV on SWD parameters

Our results demonstrated that the in utero exposure to VPA or LEV is ineffective on the development of SWDs which were recorded at PN90. Intraperitoneal VPA treatment at 200 mg/kg/day in GAERS, starting from PN5 to PN25, showed effect neither on the number nor the cumulative and mean duration of cortical discharges which were recorded several times between PN35 and PN105 [11]. In another study, treatment of WAG/Rij rats with VPA (600 mg/kg/day), starting from PN30 for 17 weeks, reduced the SWD parameters but the authors explained this effect may be due to antiseizure effect of the drug [9].

Similarly, early treatment with LEV has shown limited antiepileptogenic activity previously in GAERS and WAG/Rij rats. Treatment of GAERS with LEV at 54 mg/kg/day between PN23-60 reduced the epileptiform activity measured at PN61-64 but this effect did not persist until PN120-124 [8]. Also, in WAG/Raj rats, LEV at ~80 mg/kg/day starting from the age of 4 weeks for 17 weeks showed only a temporary antiepileptogenic effect after the treatment. However, the authors have noted that intervention at an earlier age may have a more profound effect on the epileptogenic process [12].

Early postnatal treatment with VPA or LEV was previously suggested to show antiepileptogenic effect, lasting after the treatment is stopped [1,10,30]. However, following studies revealed that this effect may not be permanent [11,12]. As opposed to the previous studies, the offsprings in our experiment were exposed to VPA or LEV in utero and our results support that VPA and LEV show antiseizure effects without antiepileptogenic effects.

4.3. The effects of in utero exposure to LEV on histopathology of the newborn GAERS

We observed that the in utero exposure to LEV has resulted in thickened alveolar epithelium in most regions, suggesting incomplete alveolar development. Despite we could not find studies of in utero exposure, LEV (70 mg/kg/day) administration caused thickened interalveolar septum, fibrosis, and chronic inflammatory cell infiltration in the lungs of adult Wistar albino rats [22]. In addition, a case-study reported occurrence of isolated eosinophilic pneumonia caused by LEV [16]. In two other cases, LEV has induced diffuse interstitial lung disease after an increase in dosage [17] and starting the drug to prevent seizures after surgery for metastatic brain tumour from lung cancer [18].

In our study, the kidneys of LEV-exposed offsprings showed dilated Bowman's spaces in some renal corpuscles, which may be interpreted as a deleterious effect on kidney. A previous study demonstrated widening of the Bowman's spaces and thickening and disruption of the glomerular basement membrane in LEV-treated (1.5 mL/day distilled water containing 36 mg LEV, intragastrically by gavage) adult pregnant albino rats weighing 200 g [31], supporting a possible LEV toxicity. Also, the scientific discussion report of European Medicines Agency [32] mentions occurrence of LEV-induced nephropathy in male Sprague-Dawley rats after 2- or 4-weeks of intravenous treatment with the drug. However, the No Observed Adverse Effect Level (NOAEL) was found to be 225 mg/kg/day both for 2- and 4-week treatments, which is a much higher dose than we used. In the only study about the effects of in utero exposure to LEV (1 mL/kg/day solution containing 25 or 50 mg/kg LEV, intragastrically by gavage), the drug did not show apoptotic effect on the kidneys of the offsprings of Sprague–Dawley rats [33]. Despite there are reports of LEV-induced acute interstitial nephritis and rhabdomyolysis cases, an observational study with a large sample size did not find an association in between [19]. The adverse effects we observed in the present study may be due to the susceptibility of the rats in the prenatal period or possible differences in strains regarding their responses to LEV.

A study comparing cerebral ventricular volumes and cortical thickness of GAERS and Wistar rats from PN10 to PN60 found that GAERS had smaller brain volumes with thinner cortical and striatal thickness [34]. In agreement with this study, our histological examination in the present study revealed irregularities in cortical thickness in unexposed naïve GAERS control and LEV-exposed newborns when compared to naïve Wistar control group. However, our finding was not statistically significant.

5. Conclusions

In conclusion, we did not find a statistically significant effect of the in utero exposure to VPA or LEV on the SWD parameters in adulthood. This may suggest that the mechanisms responsible for the observed antiepileptogenic effects in previous studies may not be operating in the prenatal period. For the histopathology part, we have observed a strong detrimental effect of in utero exposure to LEV on the lungs of the newborns, as well as a less pronounced but still significant effect on the kidneys. Further studies with advanced molecular and biochemical techniques will improve our understanding of the effects of in utero exposure to antiseizure medications throughout the pregnancy.

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CRediT authorship contribution statement

Berk Can Kantarci: Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Ahmet Sanli:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Seyhmus Gavas:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Aylin Toplu:** Formal analysis, Investigation. **Zehra Nur Turgan Asik:** Formal analysis, Investigation. **Ozlem Tugce Cilingir-Kaya:** Investigation, Writing – original draft, Writing – review & editing, Visualization. **Medine Gulcebi Idrizoglu:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing. **Feriha Ercan:** Investigation, Writing – original draft, Writing – review & editing, Visualization. **Filiz Onat:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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