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Review article

Adverse effects of prenatal and early postnatal exposure to antiepileptic drugs: Validation from clinical and basic researches

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Abstract

Epilepsy requires the long-term administration of antiepileptic drugs (AEDs), and thus, we must consider the effects of prenatal AED exposure on fetus when treating female patients of child bearing age. Large prospective clinical researches in humans have demonstrated the following: (1) prenatal exposure to valproic acid (VPA), carbamazepine, and phenobarbital increases the risk of congenital malformations in a dose-dependent manner and (2) prenatal exposure to VPA increases the risk of higher brain function impairments including intellectual disabilities and autistic spectrum disorders in the offspring. Furthermore, basic researches in animals have shown that prenatal exposure to specific AEDs causes microscopic structural abnormalities in the fetal brain. Specifically, prenatal exposure to VPA has been reported to inhibit the differentiation of neural progenitor cells during the early to middle phases of neuronogenesis, leading to increased number of projection neurons in the superficial layers of postnatal neocortices in mice. It is indispensable to prescribe AEDs that are associated with lower risk of congenital malformations and impairment of higher brain functions as well as to administer them at requisite minimum doses.

Keywords: Antiepileptic drugs; Congenital malformation; Neurodevelopmental disorders; Breastfeeding; Epigenetics

1. Introduction

Epilepsy is the most common chronic neurological condition, with a prevalence of 4–10 people per 1000 population [1,2]. Treatment for epilepsy generally requires the long-term administration of antiepileptic drugs (AEDs). Since most AEDs pass through the placenta at their specific concentrations [3], consideration of the maternal and fetal risks associated with uncontrolled seizures against the potential undesired effects from exposure to AEDs is indispensable when treating pregnant epileptic mothers [4,5]. These undesired effects include miscarriages, stillbirths, intrauterine growth retardation (IUGR), congenital malformations, and neurodevelopmental disabilities [6]. According to a report from the International Registry of Antiepileptic Drugs in Pregnancy (EURAP), an international registry that covers countries in Europe, Asia, Oceania, Latin America, and Africa, the most frequently administered AEDs during pregnancy are lamotrigine (LTG), carbamazepine (CBZ), valproic acid (VPA), and levetiracetam (LEV), accounting for approximately 80% of all AED monotherapies for epileptic mothers [7]. In North American countries, topiramate (TPM) is also frequently used in addition to the aforementioned AEDs [8].

In the late 1990s, several independent research groups established registries for epileptic mothers in an attempt to analyze large numbers of pregnancy outcomes after exposure to AEDs in a prospective manner, and are

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now reporting long-term outcomes [9–11]. Furthermore, prenatal and early postnatal AED exposure is also an important research target in the area of basic biological science, since such exposure may lead to structural and/or functional impairments, referred to as "developmental origin of health and disease (DOHaD)" [12–15].

In this article, we shall summarize our current knowledge of prenatal and early postnatal AED exposure, from both clinical and basic research aspects. Moreover, we aim to propose future research questions that may arise based on current knowledge.

2. Observations from clinical registries

Epilepsy and pregnancy registries include national registries (e.g., United Kingdom Epilepsy and Pregnancy Register [UKEPR]; Australian Register of Antiepileptic Drugs in Pregnancy [APR]), regional registries (e.g., North American AED Pregnancy Registry [NAAPR]; Neurodevelopmental Effects of Antiepileptic Drugs [NEAD] study group), and broadly international registries (e.g., EURAP). In this section, we shall discuss the outcomes of AED-exposed pregnancy based on these clinical researches, focusing especially on major congenital malformations (MCMs) and neurodevelopmental disorders. Additionally, we shall discuss early postnatal exposure to AEDs through breastfeeding, which is another important issue during the peripartum period.

2.1. Major congenital malformations

Prenatal exposure to older-generation AEDs (i.e., VPA, CBZ, phenobarbital (PB), and phenytoin (PHT)) has been widely accepted to increase the risk of MCMs to 4–10% compared with 1–5% in the general population [4,16–18] (Fig. 1). The incidence is higher when AEDs are administered (1) during the first trimester [16], (2) at high-dose [2,4,8,19], and 3) in combination with other AEDs [4,16,20].

The most common major congenital malformations in AED-exposed offspring are heart defects, neural tube defects, hypospadias, clubfoot, and cleft lip or palate [2,16]. Among these MCMs, neural tube defects are especially associated with prenatal exposure to VPA (1–5% of exposed offspring) and CBZ (0.5–1.0% of exposed offspring) [4,16].

A dose-dependency of the incidence of MCMs has been observed for prenatal exposure to VPA [2,4,8,19], CBZ [2,4,19] and PB [4]. The incidence is particularly high (23%) when VPA is administered at 1500 mg/day or higher-doses [4,8]. Thus, the International League Against Epilepsy recommends avoiding the administration of VPA to women of childbearing age [21].

There is limited data with regard to zonisamide (ZNS) that is mainly used in Japan and the United States. The NAAPR has reported that ZNS did not increase the risk for MCMs, although the study population was small and the results, thus, require future investigation [8].

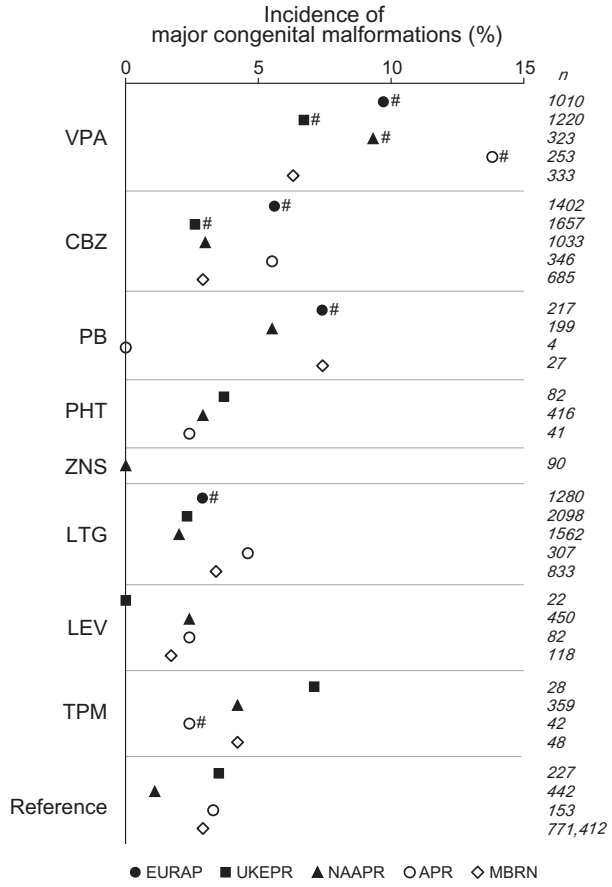

Fig. 1. Incidence of major congenital malformations after prenatal exposure to valproic acid (VPA), carbamazepine (CBZ), phenobarbital (PB), phenytoin (PHT), zonisamide (ZNS), lamotrigine (LTG), levetiracetam (LEV), topiramate (TPM), and reference. Data reported from the International Registry of Antiepileptic Drugs in Pregnancy (EURAP), closed circles [4]; United Kingdom Epilepsy and Pregnancy Register (UKEPR), closed squares [2,19]; North American AED Pregnancy Registry (NAAPR), closed triangles [8]; Australian Register of Antiepileptic Drugs in Pregnancy (APR), open circles [20]; and the Medical Birth Registry of Norway (MBRN), open squares [23]. Numbers shown to the right are the size of each study population. Note that the reference is the results of pregnancy without AED exposure, although the presence of epilepsy in the mother differs among each research group. #, dose-dependency reported.

Among the newer-generation AEDs (i.e., LTG, LEV, and TPM), LTG has been the most widely investigated. Several studies have reported that the MCM incidence after prenatal exposure to LTG is equivalent to that in the general population when administered at low-dose (less than 200–300 mg/day) [4,19,22]. These MCM incidence were reported to increase to 4–5% when LTG is administered at higher-doses (200–300 mg/day or higher) [2,4], though another study reported no dose-dependent increase [22].

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Meanwhile, results from research on prenatal exposure to LEV and TPM are still being accumulated. To date, the results show no increase of MCM incidence after LEV exposure [2,8,20,23]. In contrast, the results for TPM are a matter of controversy. Some results have shown an increased MCM incidence including microcephaly and hypospadias, and an increased risk for small for gestational age birth, whereas other results have shown no increase of MCM incidence [2,8,20,23].

As for polytherapy, VPA has been reported to increase the incidence of MCM when combined with other AEDs [2,22]. Additionally, a report from Australia has pointed out that TPM increased MCM risk when used in polytherapy [20].

2.2. Neurodevelopmental effects

The production of neurons that become distributed in the neocortices, the center of cognitive function, starts at approximately 2–4 months of gestation in humans. The produced neurons migrate through the embryonic cerebral wall and attain their laminar position during 3–5 months of gestation. Synaptic development subsequently takes place from 5 months of gestation and continues after birth [24]. Thus, the fetuses of AED-treated mothers are continuously exposed to AEDs during the period of embryonic neuronal development.

Recent research has provided certain evidence of the neurodevelopmental outcomes of children who are prenatally exposed to AEDs (Table 1). Prenatal exposure to VPA has been reported to decrease the intelligence quotient (IQ) scores of children in a dose-dependent manner, compared with that of children who were exposed to CBZ, PHT, LTG, LEV, and TPM [25–27]. Furthermore, the risk for neurodevelopmental disorders was reported to increase in children prenatally exposed to VPA, both for monotherapy (12.0%) and polytherapy including VPA (15.0%), compared with that in AED-unexposed children (1.9%), with autistic spectrum disorders (ASDs) being the most frequent diagnosis [28]. A population-based study in Denmark also reported that the risk for ASDs was higher for the children prenatally exposed to VPA (4.2%), compared with that of unexposed children (2.4%) born from epileptic mothers [29]. In contrast, studies in the UK reported that prenatal exposure to CBZ, LTG, and LEV did not increase the risks for neurodevelopment disorders or impaired language skills in the children [28,30], though another study group has pointed out adverse development scores in CBZ and LTG [31].

2.3. Effects of breastfeeding from AED-treated mothers

Breastfeeding provides nutrition, immunological protection, and mother-and-child attachment [32,33], though it may lead to the postnatal AED exposure of infants if their mothers are taking AEDs. VPA, CBZ, PB, and PHT are reported to transfer into breast milk at concentrations less than 50% of that in the maternal serum [3,34]. Conversely, LEV has been reported to transfer in a higher concentration, approximately equal to that in the maternal serum [3,35–37]. However, the serum concentration of LEV in infants was reported to be approximately 13% of that in the mother, despite the high concentration in breast milk [36].

Adverse symptoms in infants who were exposed to AEDs via breastfeeding include hypertonia, restlessness, irritability, and abnormal sleep patterns, though it is difficult to clinically distinguish such symptoms from that of AED withdrawal syndrome [3]. CBZ has been reported to cause hepatic dysfunction and LTG has been reported to cause severe apnea and hepatic dysfunction in infants when exposed via breastfeeding, though the data remain inconclusive [3,38].

On the other hand, the beneficial effects of breastfeeding have been reported on the long-term neurodevelopmental outcome of children of AED-treated mothers. The IQ scores were reportedly higher in breastfed children than in non-breastfed children at the ages of 6 years, when both groups were born from mothers taking VPA, CBZ, PHT, or LTG [39]. This positive effect of breastfeeding was predominant in the children of VPA-treated mothers, whose mean IQ was 12 points higher in the breastfed children than that in the non-breastfed children. Moreover, another group reported that breastfeeding decreased the risk for autistic traits in infants born from mothers taking AEDs including VPA, CBZ, and LTG, though the beneficial effect was not present at the age of 3 years [40].

Although the present knowledge does not provide a conclusion as to whether breastfeeding should be recommended for AED-treated mothers, we advocate that mothers should be informed of the known benefits as well as the potential risks of breastfeeding during the administration of AEDs.

3. Research in animal models and *in vitro*

Fetal exposure to AEDs during pregnancy may alter the *in utero* environment during the earliest stages of fetal development. Especially when these environmental changes cause heritable changes to DNA or chromatin structures that affect the gene expression profiles not based on the nucleotide sequences, they are called “epigenetic” changes [41]. The epigenetic mechanisms include DNA methylation, histone acetylation, and non-coding RNA, which are reported to affect cell proliferation/differentiation characteristics in developing mammalian tissues [42–45]. Indeed, (1) prenatal exposure to VPA, CBZ, LTG, and LEV has been reported to decrease the level of DNA methylation in cord blood cells in human neonates [46] and (2) VPA is known to

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Table 1
Neurodevelopmental effects of prenatal AED exposure.

	Outcome measure	Age (years)	Valproic acid	Carbamazepine	Phenytoin	Lamotrigine	Levetiracetam	Topiramate	References
Meador [25]	IQ and specific cognitive outcomes	2, 3, 4.5, and 6	Dose-dependent IQ decline, impaired verbal/nonverbal ability, memory, and executive function	No negative effects on IQ	No negative effects on IQ	No negative effects on IQ	–	–	No reference group
Baker [26]	IQ and verbal/nonverbal/spatial abilities	6	IQ decline and impaired verbal/nonverbal/spatial ability in high-dose exposure (>800 mg/day) Verbal ability was impaired regardless of dose	No impairment of IQ Reduced verbal ability	–	No impairment of IQ or specific cognitive ability	–	–	Children born to women without epilepsy
Bromley [27]	IQ and specific cognitive outcomes	5–9	Dose-dependent IQ decline, impaired verbal/nonverbal/expressive language ability	–	–	–	No negative effects on IQ or specific cognitive ability, no dose-dependence	No negative effects on IQ or specific cognitive ability, no dose-dependence	Children born to women with epilepsy without AED treatment
Bromley [28]	Diagnosis of neurodevelopmental disorders	6	Increased risk for neurodevelopmental disorders, ASD being the most frequent diagnosis	No increased risks for neurodevelopmental disorders	–	No increased risks for neurodevelopmental disorders	–	–	Children born to women without epilepsy
Christensen [29]	Diagnosis of ASD and childhood autism	4–14	Increased risk for ASD and childhood autism	No increase of ASD or childhood autism	–	No increase of ASD or childhood autism	–	–	Children born from women without AED treatment and a restricted cohort of children born to women with epilepsy not treated with valproic acid
Shallcross [30]	Motor development and language skills	3–4.5	Impaired gross motor skill, comprehension/expressive language ability	–	–	–	No impairment of development or language skills	–	Children born to women without epilepsy
Veiby [31]	Motor development, language skills, and traits for neurodevelopmental disorders	1.5 and 3	Impaired gross motor skills at age 1.5 Impaired sentence skills at age 3	Impaired fine motor skills and social skills at age 1.5 Increased aggressive symptoms at age 3	–	Impaired sentence skills and increased autistic traits at age 3	–	–	Children of parents without epilepsy

AED, Antiepileptic drug; ASD, autistic spectrum disorder; IQ, intelligence quotient.

inhibit the activities of histone deacetylases (HDACs) which regulate the transcription of genes by altering the chromatin structure [47–49].

In this section, we shall discuss the current knowledge regarding exposure to AEDs during embryogenesis based on basic biological research, including both their epigenetic and non-epigenetic effects. We will focus on the most frequently administered AEDs during pregnancy, i.e., VPA, CBZ, LTG, and LEV [7] (Table 2).

3.1. VPA

Similar to observations in humans, a series of previous studies have reported that prenatal exposure to VPA causes autistic behaviors in rodent offspring. Such autistic behaviors include decreased social interactions, decreased sensitivity to pain, increased sensitivity to nonpainful stimuli, repetitive/stereotypic-like activity, increased anxiety, abnormally high and long lasting fear memories, and changes in ultrasonic vocalizations of the pups [50,51]. A relative increase in the ratio of excitatory/inhibitory synaptic function has been proposed to be the pathogenetic mechanism [52].

As for structural anomalies within the central nervous system, prenatal exposure to VPA is known to induce neural tube defects [53], neuronal migration defects leading to the impairment of neocortical lamination [54], increased weight of the whole brain [55], increased density of prefrontal neocortical neurons [56], apoptosis in the developing embryonic cerebral wall [57], and reduced adult neuronogenesis in the hippocampi [58].

It is important to note that these previous observations may not represent the effects of prenatal exposure to VPA in humans, since VPA was administered to pregnant rodents at high-dose (generally 400–500 mg/kg/-dose; approximately twice the median effective dose (ED_{50}), see Table 1), in a single or in multiple injections during the middle phase of embryogenesis, that is, usually on embryonic day 11 (E11) or E12, despite the short half-life of VPA in rodents (1–2 h) [53]. Thus, in more recent analyses, VPA was administrated to pregnant rodents at lower dosages for longer periods so as to reproduce the *in utero* environment in human epileptic mothers taking VPA.

Daily intraperitoneal (i.p.) injections of VPA to mothers (20 or 100 mg/kg/day, from E12.5 to postnatal day 23 (P23)) increased the thickness of the frontal neocortices by increasing the number of neurons in VPA-exposed rats, compared with that in controls [59]. Furthermore, we reported that the low-dose peroral administration (p.o.) of VPA throughout the whole pregnancy period (0.4% VPA water solution given as drinking water from E1 until birth) resulted in maternal plasma concentrations of 20–40 μg/ml, or approximately one-fifth of the level that increases the seizure threshold in mice (TIC_{50}) [60]; see Table 3, and increased the number of projection neurons as well as the neocortical thickness

Table 2
Structural and neurofunctional effects of prenatal AED exposure on embryos and offspring.

	Basic studies in rodents		Clinical studies in humans	
	Structural	Neurofunctional	Structural	Neurofunctional
Valproic acid	Neural tube defects [53] Apoptosis in embryonic cerebral wall [57] Increased neocortical neurons [59,61] Increased expression of cell cycle regulatory proteins in NPCs [61]	Autistic behaviors [50–52]	Dose-dependent increase of MCM incidence [2,4,8,19,20] Thickening of neocortices [62]	Dose-dependent decrease of IQ scores [25–27] Increased risk of neurodevelopmental impairment [28–31]
Carbamazepine	Decreased neuron number in hippocampi and neocortices [71] MCM risk is CI [67–70]	No impairment of learning or memory functions [71]	Increased MCM incidence, possibly dose-dependent [2,4,19]	CI [28,29,31]
Lamotrigine	Neocortical and hippocampal malformations due to impaired neuronal migration [72] Increased MCM, IUGR, and lethality of embryos in extremely high-dose exposure [74]	Hyperactive behavior due to decreased GABA-A receptor [73]	No increase of MCM incidence in low-dose administration (<200–300 mg/day). Dose-dependency is CI [2,4,19,22]	CI [25,26,28,29,31]
Levetiracetam	No induction of neocortical or hippocampal malformation [72]	No negative effects on physical or cognitive functions [75]	No increase of MCM incidence [2,8,20,23]	No impairment of IQ scores or neurodevelopmental skills [27,30]

The table presents a summary of structural and neurofunctional effects of prenatal AED exposure on embryos and offspring observed in basic and clinical studies. Note that the extrapolation of results from animal studies to humans requires sufficient evaluation because of potential interspecies variation in the effects of prenatal AED exposure. NPCs, Neural progenitor cells; MCM, major congenital malformation; CI, controversial issue; IUGR, intrauterine growth retardation.

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Table 3
Anticonvulsant activity of AEDs against seizure induction by electrical or chemical stimulation in mice.

Compound	ED_{50} (mg/kg)			TIC_{50} (μg/ml)
	6 Hz	MES	PTZ	PTZ
Valproic acid [60,79,80]	126	235–272	120–220	120–150
Carbamazepine [79,80]	47.9	7.81–8.81	>50	
Lamotrigine [80]	>60	7.47	>40	
Levetiracetam [80]	19.4	>500	>500	

ED_{50}, median effective dose; TIC_{50}, plasma AED concentration which increases the threshold for seizures by 50%; 6 Hz, seizure induction by 6 Hz stimulation; MES, maximal electroshock; PTZ, picrotoxin. Administration of each AED was conducted intraperitoneally.

in the superficial layers of the somatosensory area in the postnatal mice [61]. Additionally, we reported that prenatal exposure to VPA more than doubled the number of neurons born during the terminal phase of neuronogenesis due to an increase of NPCs in the ventricular zone of the developing cerebral wall, which was caused by a decreased quiescence (Q) fraction of the NPCs during the early to middle phases of the neuronogenetic period [61]. This result was consistent with a previous clinical study in humans in which prenatal exposure to VPA increased the neocortical thickness in children [62], and an *in vitro* study in which VPA exposure increased the number of superficial neocortical neurons born from cultured murine embryonic stem cells [63].

Furthermore, our results showed that prenatal exposure to VPA increased the amount of cell cycle regulatory proteins in the nuclei of NPCs in a nonspecific manner, including cyclin D1, cyclin dependent kinase (cdk) 2, cdk4, and cdk inhibitor $p27^{Kip1}$ [61]. These disorganized increases in protein expression were suggested to be a result of the HDAC inhibitory activity of VPA, since the amount of total acetylated histone H3 protein was increased in the cerebral walls of the VPA-exposed embryos. However, VPA has various biological functions in addition to its action as an HDAC inhibitor: VPA increases the concentration of gamma aminobutyric acid (GABA), prolongs the recovery of voltage-activated Na^+ channels from inactivation, reduces T-type Ca^{2+} currents [64], activates the glycogen synthase kinase-3β/β-catenin pathway [55], activates the extracellular signal-regulated kinase pathway [65], and decreases protein kinase C [66]. Thus, the observed phenotypes may be a mixture of these various effects overall.

3.2. CBZ

Several studies have reported that prenatal exposure to CBZ increased the incidence of MCMs in offspring, including cleft palate, enlarged cerebral ventricles, clubfoot, and skeletal defects in mice [67,68]. Additionally, prenatal exposure to CBZ has been reported to increase the risks for edema, cardiac ventricular septal defect, gastroschisis, right hydronephrosis, and omphalocele in a dose-dependent manner, in rat embryos [69]. In contrast, another study has reported no evidence of teratogenicity in mice prenatally exposed to CBZ [70].

Recent research has reported that prenatal exposure to CBZ decreased the number of neurons in the hippocampi and neocortices of postnatal mice [71]. In this study, female mice were given pellets that included 3.5 g/kg of CBZ or normal pellets as a control throughout the pregnancy period. The numbers of neurons were decreased in the hippocampi and the neocortices by 50% and 25%, respectively, in the CBZ-exposed postnatal mice, as compared with controls. However, the CBZ-exposed postnatal mice did not show any impairment in learning or memory functions [71]. Since the underlying mechanisms of the reported dysgenesis of the brain induced by prenatal exposure to CBZ have not yet been elucidated and CBZ is the second most frequently administered AED as a monotherapy during pregnancy in humans, further studies are strongly recommended.

3.3. LTG

Prenatal exposure to LTG (5–20 mg/kg/day, i.p., from E14 to E19) has been reported to dose-dependently increase the number of neuron-depleted areas in the neocortices and the hippocampi of rat offspring, which was a result of impaired neuronal migration [72]. Furthermore, another group reported that prenatal to early postnatal exposure to LTG (11–46 mg/kg/day, p.o., from E3 to P11) showed hyperactive behavior and decreased GABA-A receptor expression in the neocortices, compared with controls in rats [73]. Additionally, LTG administration at higher-doses (50–200 mg/kg/day, i.p., on E7 or E8) reportedly increased the incidence of maternal mortality, abortion, embryonic lethality, MCMs, and IUGR, compared with controls [74]. The observed malformations included maxillary-mandibular hypoplasia, exencephaly, cleft palate, median facial cleft, urogenital anomalies, and caudal regression. However, the administration dosage used in this study was extremely high, compared with the ED_{50} dose; thus, it may not be applicable to evaluating the risks of prenatal exposure to LTG (Table 3).

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3.4. LEV

Currently, prenatal and early postnatal LEV exposure has only been tested in a few animal studies, none of which have shown abnormalities in cognitive function or in the structure of the brain. Prenatal exposure to LEV (50 mg/kg/day, i.p., from E14 to E19) did not increase the number of neuron depleted areas in the neocortices [72]. Additionally, LEV (25–100 mg/kg/day, p.o., from E1 to E18) was reported to have only a transient effect on reflex maturation and no impact on physical or cognitive functions in rat offspring [75]. Postnatal exposure to LEV (250–1000 mg/kg/day, i.p., on P7) did not induce neuronal cell death in the neocortices, the hypothalamus, or the hippocampi in rats [76]. Additionally, patch-clamp recordings of medial striatal spiny neurons showed that postnatal exposure to LEV (400 mg/kg/dose, i.p., on P7) did not disrupt synaptic development in rats [77]. Since LEV is a potential candidate for first-line AED for epileptic pregnant mothers, future evaluation of its safety is highly expected.

4. Conclusion

Recent clinical research has revealed that several AEDs indeed affect embryonic development, and some observations from animal studies have indicated the possible pathogenic mechanisms of these phenotypes observed in humans. However, we should not apply the results of animal studies to humans without sufficient evaluation. The short life spans, large litter sizes, and different patterns of AED metabolism among species often make animal models poor proxies for human embryos [78]. At least, we can say that different types of AEDs may have unique effects on the development of the brain, of which their underlying mechanisms and their impact on postnatal higher brain functions would be our future study questions.

Again, the administration of AEDs is an indispensable treatment for adult patients with epilepsy, and such treatment is necessary throughout life for more than half of all patients. Thus, we should be aware of the risks and benefits of taking AEDs, especially during pregnancy. However, there are certain circumstances in which the prescription of high-risk AEDs such as VPA is inevitable, for instance, in the treatment of juvenile myoclonic epilepsy. In such cases, the risks for MCMs and higher brain function impairments can be lowered by (1) decreasing the administration dosage with careful validation of serum concentration, (2) prescribing extended-release tablets, and (3) avoiding the use of multiple AEDs in combination. Furthermore, several newer-generation AEDs have been developed in recent years and we, therefore, expect that safe and effective AEDs will be available in the near future.

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