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ORIGINAL PAPER

Effects of lacosamide "a novel antiepileptic drug" in the early stages of chicken embryo development

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Abstract

Introduction Antiepileptic drugs (AEDs) are teratogens and confer a risk of congenital malformation. The estimated prevalence of major congenital malformations such as cardiac defects, facial clefts, hypospadias, and neural tube defects in epileptic women is 4–10 %, which represents a two- to fourfold increase in pregnant women compared to the general population. However, there are no clear data for newer drugs. Lacosamide (LCM), a novel AED, is the first of the third-generation AEDs to be approved as adjunctive therapy for the treatment of partial-onset seizures. There are no data on the pharmacokinetics of LCM during pregnancy, and only some published data have reported on its effects during pregnancy.

Methods In this study, three different doses of LCM (0.12, 0.5, and 1.60 mg in 0.18 mL) were applied under the embryonic disks of specific pathogen-free Leghorn chicken embryos after a 30-h incubation. Incubation was continued for 80 h, at which time all embryos were evaluated macroscopically and microscopically.

Results There was growth retardation in all of the LCM-treated groups. Major malformations increased in a dose-dependent manner and were mostly observed in the supratherapeutic group.

Conclusion Based on our data, LCM may cause growth retardation or major congenital malformations. Nevertheless, more extensive investigations of its reliability are needed.

Keywords Anti-epileptic · Chick embryo · Lacosamide · Malformation

Introduction

In the USA, over 1 million women of childbearing age have epilepsy and the continued use of antiepileptic drugs (AEDs) is recommended to reduce the maternal and fetal trauma associated with seizures [1]. However, prenatal exposure to AEDs can cause major congenital malformations, growth retardation, and intelligence deficits in the developing fetus [1–3]. The estimated prevalence of major congenital malformations, such as cardiac defects, facial clefts, hypospadias, and neural tube defects, in the children of epileptic women is 4–10 %, which represents a two- to fourfold increase compared to the general population [3]. In this group of patients, the treatment target with mono- or polytherapy should be optimal seizure control with minimum fetal exposure to AEDs.

The risks of the AEDs valproic acid, phenytoin, phenobarbital, levetiracetam, oxcarbazepine, and topiramate have been reported [1–4]. The North American Antiepileptic Drug Pregnancy Registry assessed the risk of major congenital malformation with AED exposure as 9.3 % for valproate, 5.5 % for phenobarbital, 4.2 % for topiramate, 3.0 % for carbamazepine, 2.9 % for phenytoin, 2.4 % for levetiracetam, and 2.0 % for lamotrigine [4]. However, there are limited data for newer drugs.

Lacosamide (LCM), a novel third-generation AED, was first approved as adjunctive therapy for the treatment of partial-onset seizures in 2008 and for monotherapy in 2014 [5]. It has been suggested that LCM is a safe, effective, well-

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Table 1 Numbers and percentages of normal and abnormal embryos after incubation with physiological saline and varying amounts of LCM

Groups	Embryos n (%)	Lethal n (%)	Observed n (%)	Growth retardation n (%)	Malformation n (%)
Sham	7 (100 %)	0 (0)	7 (100 %)	7 (100 %)	0 (0)
Subtherapeutic	12 (100 %)	4 (33 %)	8 (66 %)	7 (58 %)	1 (8 %)
Therapeutic	11 (100 %)	3 (27 %)	8 (72 %)	5 (45 %)	3 (27 %)
Supratherapeutic	12 (100 %)	2 (16 %)	10 (83 %)	5 (41 %)	5 (41 %)

tolerated adjunctive treatment for reducing seizure frequency in patients with highly refractory partial seizures [6]. For seizures, its recommended daily dose is 200–600 mg [7]. However, there are no data on the pharmacokinetics of LCM during pregnancy and only two reports on its effects during pregnancy [8, 9]. Therefore, we examined the effects of 0.12 (subtherapeutic), 0.5 (therapeutic), and 1.6 (supratherapeutic) mg doses of LCM on chick embryos in ovo.

Material and methods

This study was conducted with the cooperation of the Histology Department Research Laboratory of Celal Bayar University Medical School. Fertilized, specific-pathogen-free Leghorn chicken eggs were supplied by the Republic of Turkey Ministry of Agriculture and Rural Affairs, Bornova Veterinary Control and Research Institute. All experiments were conducted in accordance with the animal research protocol of Celal Bayar University Ethics Committee (no. 77.637.4335–27).

Incubation and injection

Forty-two eggs weighing 65 ± 5 g (mean $\pm$ SD) were incubated at 37.5 ± 0.2 °C and 60–80 % relative humidity. Each egg was repositioned on its axis every 2 h. After 30 h of incubation, each egg was opened under ×4 optical magnification [10–12], when at Hamburger–Hamilton stage 9 [11]. They were rinsed with 70 % ethanol, a piece of plastic tape was placed close to the egg air cavity, and a small hole was opened for injections. In each group, the embryonic disks were identified and the same volume of liquid (total 180 μL) was injected under each disk with a 30-gauge syringe.

Drug preparation

Intravenous LCM solution is available in a 10-mg/mL preparation. LCM solutions of three concentrations were prepared. The doses given were calculated according to the weight of the eggs with reference to the daily dose range used in humans. In the LCM-treated groups, 12, 50, or 160 μL LCM solution was diluted with physiological saline to a total volume of 180 μL.

Groups

The eggs were divided into sham (group 1, $n = 7$) and LCM-treated ($n = 35$) groups. The LCM-treated group was subdivided into three groups according to the drug dose: 0.12 mg (subtherapeutic group, $n = 12$), 0.5 mg (therapeutic group, $n = 11$), and 1.6 mg (supratherapeutic group, $n = 12$). In all of the groups, the eggs were closed with sterile tape after injection and incubation was continued for 80 h, at which time the eggs were reopened and the embryos were dissected from the embryonic membranes with adherence to microsurgical rules, using the water-floating technique. Then all embryos were put into a 10 % formalin solution for 24 h. The embryos were viewed under an Olympus (SZX7) stereomicroscope.

Results

Embryos from the sham and treatment groups were observed and photographed macroscopically and
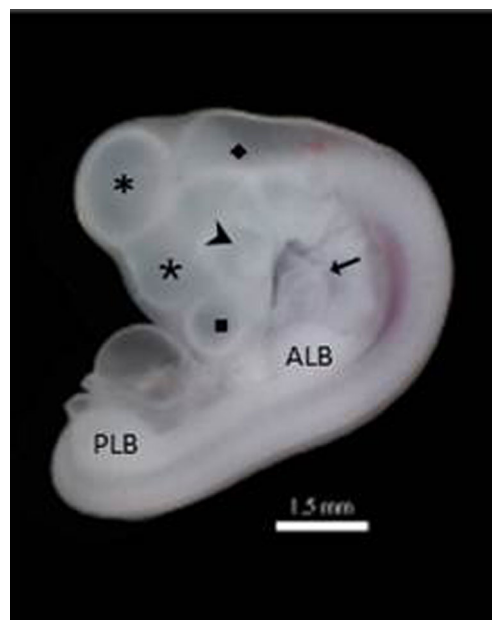

Fig. 1 Normal development of chick embryo after physiological saline solution injection. Telencephalon (*black square*), diencephalon (*black star*), mesencephalon (*asterisk*), metencephalon (*black diamond suit*), heart (*triangle-headed rightwards arrow*), eye (*black rightwards arrowhead*), *ALB* anterior limb bud, *PLB* posterior limb bud
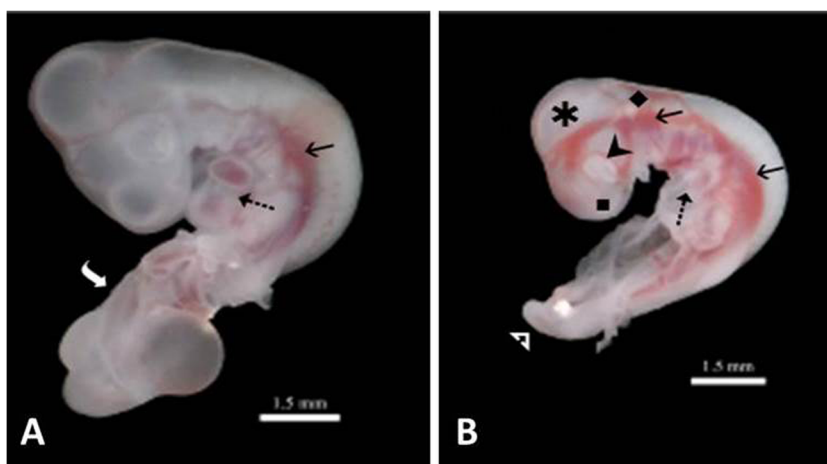
Fig. 2 **a** and **b** demonstrated the subtherapeutic group of embryos with growth retardation and major malformations after LCM injection, respectively. Growth retardation in brain vesicles (small telencephalon (*black square*), mesencephalon (*asterisk*) and metencephalon (*black diamond suit*)),shrinkage in eyes/microphthalmia (*black rightwards* *arrowhead*), anomaly in heart development (*rightwards dashed arrow*), excessive growth and expansion in blood vessels (*triangle-headed rightwards arrow*), super-twisted body (*shrinkage*) (*black curved downwards and rightwards arrow*), short tail (⯈)

microscopically. Table 1 summarizes the characteristics of the normal and abnormal embryos after incubation with varying amounts of LCM and physiological saline.

In the sham group, no growth retardation or major congenital malformation was detected in any of the seven embryos (Table 1, Fig. 1). In the subtherapeutic group, 4 of 12 embryos died during the procedure. Of the remaining 8, 7 showed growth retardation and 1 had a major malformation (Table 1, Fig. 2). In the therapeutic group, 3 of 11 embryos died during the procedure. Of the remaining 8, 5 showed growth retardation and 3 had major malformations (Table 1, Fig. 3). In the supratherapeutic group, 2 of 12 embryos died during the

procedure. Of the remaining 10, 5 had growth retardation and 5 had major malformations (Table 1, Fig. 4).

Discussion

The pharmacological treatment of epilepsy during pregnancy is problematic. It is necessary to balance the potential teratogenic effects of AEDs on the fetus with the irreversible damage of uncontrolled epilepsy done to the mother and fetus in the management of epilepsy during pregnancy [10]. Complications related to seizures include fetal death, poorer
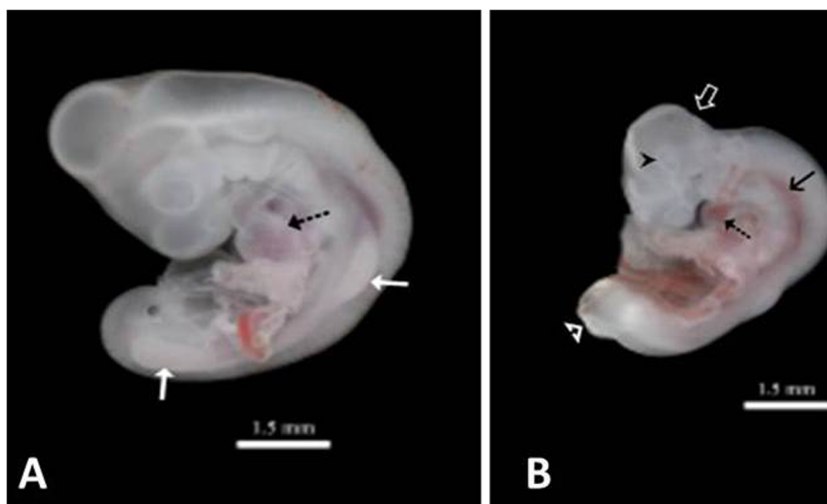

Fig. 3 **a** and **b** demonstrated the embryos with growth retardation and major malformations in therapeutic group after LCM injection respectively. Anencephaly (*rightwards white arrow*), shrinkage in eyes/microphthalmia (*black rightwards arrowhead*), abnormal heart looping (*rightwards dashed arrow*), excessive growth and expansion of blood vessels (*triangle-headed rightwards arrow*), reduced size of limbs (*triangle-headed rightwards arrow*), short tail (⯈)
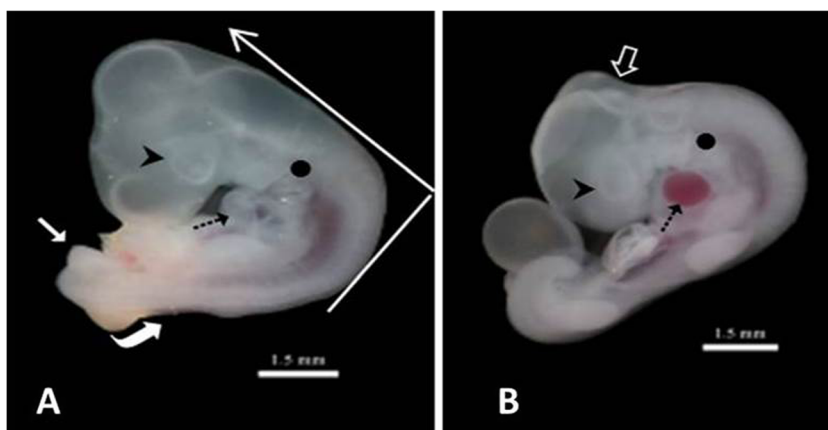
Fig. 4 a and **b** demonstrated the embryos with growth retardation and major malformations in supratherapeutic group after LCM injection, respectively. Anencephaly (*rightwards white arrow*), shrinkage in eyes/ microphthalmia (*black rightwards arrowhead*), anomaly in heart development (*rightwards dashed arrow*), shortening and thickening of the body (*↗*), reduced size of limbs (*triangle-headed rightwards arrow*), reduction in vascularization (*black circle*), super-twisted body (*shrinkage*) (*black curved downwards and rightwards arrow*)

cognitive development, preterm labor, and maternal injury. Congenital malformations (such as cardiac defects, facial clefts, extremity abnormality, neural tube defects) and growth retardation could be seen related to the teratogenic effects of AEDs [2, 13, 14]. Therefore, treatment requires extra care and the goal should be to control generalized tonic-clonic seizures with minimal in utero fetal AED exposure [1, 13].

Many AEDs have been reported to have teratogenic effects; these include carbamazepine, gabapentin, lamotrigine, levetiracetam, phenobarbital, phenytoin, topiramate, and valproate. Lamotrigine and levetiracetam appear to confer low risks for both anatomical and behavioral teratogenesis [15]. However, less is known about the teratogenic effects of newer AEDs, which necessitates studies in animal models due to the limitations inherent in human epidemiological and clinical AED studies. In humans, pregnancies exposed to AEDs in the first trimester are at increased risk of major congenital malformations. The early chick embryo model is an ideal model that corresponds to the first month of embryonic development in mammals. It is also suitable for investigating the effects of chemical agents on embryo development [10]. Therefore, we observed chick embryos at 80 h of development after injecting LCM and physiological saline solution.

LCM is a newer AED with a dual mode of action. It selectively enhances the slow inactivation of voltage-gated sodium channels without affecting fast inactivation, and modulates collapsing response mediator protein 2 (CRMP-2) [6, 16]. CRMP-2 is a part of the signal transduction cascade of neurotrophic factors and has neuroprotective effects. The ability of LCM to modulate CRMP-2 contributes to the decreased neuronal loss observed in status epilepticus [6]. It is also efficacious for treating neuropathic pain and neuroprotection [17, 18].

The Food and Drug Administration has classified LCM as a human pregnancy class "C" compound [9], which means that animal reproduction studies have shown an adverse effect on the fetus and there are no adequate, well-controlled studies in humans, but the potential benefits may warrant use of the drug in pregnant women despite the potential risks. A literature review of PubMed found only one case study and one experimental study that have reported its effects during pregnancy [8, 9]. Ylikotila et al. treated a 7-week-pregnant woman who had cerebral venous thrombosis and status epilepticus with a combination of LCM and levetiracetam and reported that the infant was born without malformations, but was small for gestational age [8]. In their case study, the antiepileptic treatment was started in the late organogenesis period [8]. In an experimental study, Lee et al. [9] investigated the teratogenic potential of LCM using a zebrafish model and reported that LCM induced head and tail malformation, scoliosis, and growth retardation, and was teratogenic; in addition, there were significant differences among dose levels. Our study examined chicken embryos at the Hamburger and Hamilton [11] stage coinciding with 80 h of embryogenesis. In the LCM-treated groups, although most of the embryos appeared normal, growth retardation was obvious. The growth retardation was the least in the subtherapeutic group, while major malformations increased with the dose and were mostly observed in the supratherapeutic group (Table 1).

Conclusion

Based on our data, LCM is not safe for developing embryos and may cause growth retardation or major congenital malformations. Nevertheless, more extensive investigations of its reliability are needed.

Compliance with ethical standards All experiments were conducted in accordance with the animal research protocol of Celal Bayar University Ethics Committee (no. 77.637.4335–27).

Conflict of interest Authors declare that there is no conflict of interest.

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