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Evaluation of Teratogenic Activity of Antiepileptic Drug Lamotrigine in Mouse Fetuses

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Background: Use of antiepileptic drugs during pregnancy can be associated with an increased risk of teratogenicity as well as congenital abnormalities. However, there are numerous discrepancies to determine whether lamotrigine, as an antiepileptic drug, can significantly induce malformation in newborn infants or not. Thus, the purpose of the study was to evaluate the teratogenic effects of lamotrigine on mouse fetuses.

Materials and methods: In the present study, 21 pregnant mice were assigned to four groups. Groups 1 and 2 (controls) received mock treatment and ethanol 20%, respectively. Groups 3 and 4 (treatment) were intraperitoneally administered with 25 and 75 mg/kg lamotrigine for three days, respectively. The treatment protocol was performed within the gestational days of 9-18 in all groups. On gestational day 18, 117 fetuses were taken out of the fallopian tube of studied mice and then examined for any anomalies (vertebral, limbs and cranial), followed by a measurement of their height and weight.

Results: The results revealed that, in the treated groups, the weight and the height had significantly decreased ($p<0.01$) and also various anomalies were evident. Moreover, as the dose of lamotrigine increased, the decrease in the weight and the height and rising trend in anomalies were intensified.

Conclusion: According to the findings, lamotrigine (LTG) could be considered as a risk factor for the development of the anomalies examined.

BACKGROUND

Birth defects, congenital deformities, congenital anomalies, as well as congenital abnormalities are terms similarly referred to deficiencies in structure, function, and metabolism leading to physical and mental disabilities or death.[1] Genetic and environmental factors as well as their potential interactions involved in pathogenesis include single gene disorders, chromosomal abnormalities, maternal medical conditions, and exposure to environmental toxins, substance abuse, infections, drugs, chemicals, radiations, and hyperthermia. The mechanical constraints on fetal[2] and antiepileptic drugs (AEDs) have been reported to affect fetal development throughout pregnancy and also increase the risk of major congenital abnormalities in the neonates[3,4].

According to previous reports, traditional antiepileptic drugs such as phenobarbital, phenytoin, carbamezapine, and valproate can increase the risk of congenital malformations in pregnant women.[3,5] Generally, AEDs are classified into two groups according to their risks for pregnancy. The first group consists of a number of conventional drugs that have been extensively investigated for teratogenic effects. The second group, as the newer AEDs, has not been confirmed in terms of their teratological effects.[6,9] In general, LTG can have an acceptable safety profile and it can be used as an effective medication in the treatment of epilepsy and various cognitive disorders.[10,11] For example, $C_9H_7C_{12}N_5$ is a phenyltriazine derivative that is rapidly and completely absorbed after oral administration.[12,13] This drug can also act by inhibiting the release of glutamate in voltage-dependent sodium channels.

There are few studies regarding the emergence of LTG-induced teratogenic anomalies in pregnancy and epilepsy.[3,14] Moreover, there are several methodological challenges concerning such investigations. For example, a small sample size inadequately demonstrates possible teratogenic effects of AEDs, namely lamotrigine. Furthermore, comprehensive interpretations of teratological data have not been evaluated in previous studies. Nevertheless, some research on animals have shown that oral administration of LTG in mice and rats induces no teratogenic effects, but it can increase preterm birth and fetal death.[14] In another study by Bastaki et al.[15], LTG at 25-300 mg/kg in pregnant mice was reported to initiate muscle contraction, weight loss, preterm birth, as well as craniofacial deformities such as cleft palate and exencephaly. However, no vertebral and limb anomalies were reported. In the brain of rat fetuses receiving lamotrigine, a reduced body weight at birth could increase the volume and the diameter of the cerebral structure whilst augmented density of subcortical layer and ventricle dilatation was reported.[16] No study has been inclusively conducted to examine the teratogenic effects of LTG in humans and most investigations have shed light on LTG in combination with other drugs. Therefore, the observed fetal complications could not be attributed merely to LTG. So far, a few research studies have been conducted to explore the physiological mechanisms of LTG and its potential therapeutic applications in mice models of epilepsy[17,19] but there are limited studies on LTG teratogenicity. Additionally, most studies have considered oral intake of the drug. Therefore, the purpose of this study was to investigate the in vivo teratogenic effects of intraperitoneal administration of LTG on mice fetuses. Such studies could help further understand the negative effects of teratogenes in terms of preventing the potential side effects on pregnant women and fetuses.

MATERIALS AND METHODS

This experimental study was conducted on 21 pregnant mice (Pasteur Institute, Tehran, Iran). The use of animals in this experiment was approved by the Research Committee affiliated to Department of Anatomy, School of Health, Shahrekord University of Medical Sciences, under the guidelines of the National Advisory Committee for Laboratory Animal Research[20] and National Institute of Health for the use of laboratory animals.[21] After the animals were transferred to the School of Medicine at Shahrekord University of Medical Sciences, they were allowed to adapt themselves to the new environment at 21±1°C and 12 h light/dark cycle. Then, three or four female mice were kept with one male mouse in a cage for breeding. The next morning, the mice were examined for vaginal plug and the ones with a vaginal plug were isolated. To closely determine the onset of pregnancy, the researchers separated male and female mice again and the day the vaginal plug was noted was considered as day 0 of pregnancy.

It should be noted that pure LTG powder (Hetero Drugs Limited, India) is slightly soluble in water, so LTG was dissolved in ethanol.[22] Ethanol 20% (Merck, Germany) modified with 1 ml distilled water was used for LTG administration. Since ethanol by itself is a potential confounding factor, a control group (control 2) was considered which was administered with ethanol alone.

It has been established that LTG at a dose of 25 mg/kg can act as a teratogen and it can also lead to the death of mice at doses higher than 75 mg/g.[18] Therefore, a dose range of 25-75 mg/kg was considered for the present study. The pregnant mice were then assigned to four groups; group 1 (control one; 32 offspring obtained) received no treatment, group 2 (control two; 31 offspring obtained) was intraperitoneally administered with 1 ml ethanol three times a day (every 8 hours) from the gestational day 9, and groups 3 (treatment one; 36 offspring obtained) and 4 (treatment two; 18 offspring obtained) were intraperitoneally administered with LTG at 25 and 75 mg/kg doses, respectively.

On gestational day 18, all the mice were anesthetized and killed through cervical dislocation. Then, the embryos were taken out of the fallopian tube by opening an anterior abdominal wall and separately examined for superficial morphology, as well as vertebral, limb, and cranial anomalies upon the removal of fetal membranes. Three types of anomalies consisting of vertebral (scoliosis and kyphosis), limb (amelia and micromelia), and cranial (exencephaly and anencephaly) were evaluated and the incidence rates of resulting complications for the four studied groups were compared. The weight of fetuses was then measured using a digital weight (Sartorius) to the nearest 0.01 g and their height (crown-rump length) was measured using a caliper to the nearest 0.1 mm. The obtained data were analyzed by Kruskal-Wallis H test and Chi-square test using SPSS Statistics (Version 22) and levels of significance were considered by <0.05.

RESULTS

The findings of the study are summarized in **Table 1. Figs 1** and **2** also illustrate some emerged anomalies in the formation of fetal extremities. Based on the findings, ethanol caused a decrease in weight and height. Once LTG was introduced, a significant descending trend in weight and height was also observed, that was further increased by adding the dose ($p<0.001$). The incidence rates of anomalies among the four groups were also compared in **Table 1**. In groups 3 and 4, the incidence rates of anomalies increased as the dose of LTG was administered. **Table 2** shows the number and percentage of observed skeletal anomalies in mouse fetuses in the four study groups. Anomalies were observed in the sham group (receiving only the solvent) and treatment groups but the difference between the sham group and treatment groups was reported to be significant ($p< 0.05$). Notably, 5 min after administration of LTG at 75 mg/kg, myoclonic contractions were initiated and continued for 2 to 3 minutes. During pregnancy, following the administration of lamotrigine, 1 and 3 deaths in the 25 and 75 mg/kg groups were observed, respectively.

DISCUSSION

Treatment of epilepsy in pregnant women can be consistently associated with many complications, so fetuses exposed to AEDs are more predisposed to various problems.[15,23] Lamotrigine, one of the novel anti-epileptic medications, is widely used either alone or in combination with other medications for treating epileptic seizures in adults and children. There is also enough evidence showing that LTG passes through the placenta easily and is secreted in the breasts.[24] Moreover, LTG is classified as a group C drug and its use is limited to the treatment of various types of epilepsy according to Food and Drug Administration.[24] However, since this medication can contribute to the treatment of other chronic neurological diseases including bipolar disorder and migraine with aura, trigeminal syndrome is increasing.[11,25,26] Given that the available data regarding teratogenic effects of LTG are scarce, previous studies have mostly considered oral intake of this drug.[3,27] Furthermore, it is increasingly being taken for the treatment of many neurological diseases. Thus, the purpose of this study was to investigate the *in vivo* teratogenic effects of LTG on mice fetuses.

The study of superficial characteristics and anomalies in the fetuses of the mice administered with LTG have indicated some variations in the course of growth and development as well as morphogenesis of these fetuses (**Table 1**). As shown in

Table 1. Comparison of height, weight, and anomalies in mice fetuses among different groups. p-values indicate comparison between treatment groups (receiving 25 and 75 mg/kg lamotrigine) and control 2 (sham) group

Study group / Variable	Control (n=32)	Sham (n=31)	Treatment 1 (n=36)	P-value	Treatment 2 (n=18)	p-value
Median weight (g)	1.38	0.99	0.865	<0.001	0.84	<0.001
Median height (mm)	2.615	2.04	2	<0.05	1.795	<0.001
Rate of anomalies (%)	0.0%	16.1%	50%	<0.001	52.8%	<0.001

Table 2. Frequency and percentage of observed skeletal anomalies in mice fetuses of four study groups

Study group / Variable	Control* (n=32)	Sham# (n=31)	Treatment 1 (n=36)	P-value	Treatment 2 (n=18)	p-value
Scoliosis	0	2 (6.5 %)	13(36.1%)	<0.001	7(38.9%)	< 0.001
Costal fusion	0	5 (16.1 %)	11(30.6%)	<0.05	6(33.3%)	<0.05
Bifurcation	0	4 (12.9%)	11(30.6%)	<0.05	6(33.3%)	<0.05

*Mice in the control group did not receive anything.
#Mice in the sham group received only the solvent.

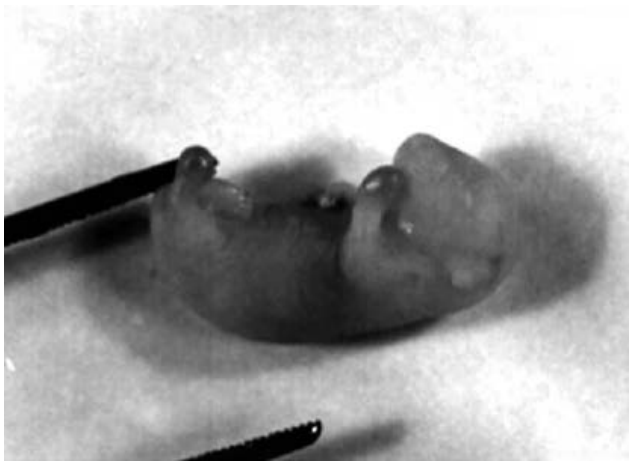

Figure 1. Anomalies in the formation of fetal extremities in the embryo administered with lamotrigine at 25 mg/kg.

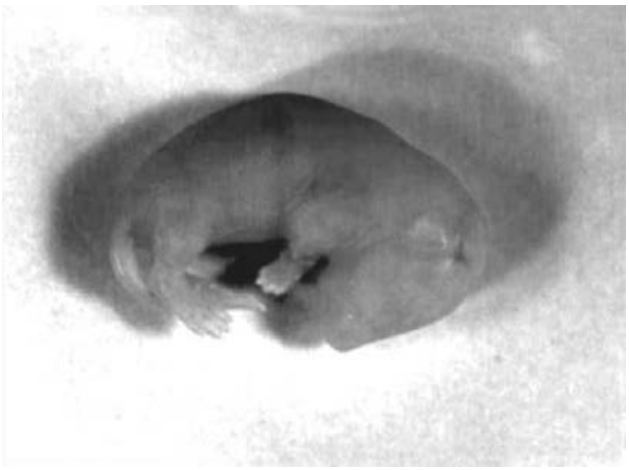

Figure 2. Kyphosis and aplasia of the right upper limb in the embryo administered with lamotrigine at 75 mg/kg.

Table 1, there was a significant difference in terms of height and weight among the four study groups. In group 2, a decrease in weight and height after administrating ethanol as a LTG solvent was seen compared to group 1.

Consistent with previous investigations,[7,15,28] the findings of the present study indicated that the height and the weight of the fetuses had significantly decreased once LTG had been administrated in a dose-dependent manner (**Table 1**).

Decreased height and weight could be due to a variety of reasons, but researchers generally argued that the use of AEDs during pregnancy could cause decreases in total protein content within the fetuses and consequently a decline in height and weight.[27] Obviously, LTG during pregnancy could be considered as a risk factor for fetal growth and development whilst potentially causing reductions in the weight and height of the fetuses. The obvious anomalies in the present study included scoliosis and kyphosis (in vertebrae), amelia and micromelia (in limbs), as well as unencephaly and exencephaly (in cranium). Based on the findings of this investigation, various anomalies increased in group 2 in comparison with group 1, but the incidence rate of anomalies significantly increased with the addition of LTG in a dose-dependent manner in groups 3 and 4. There are also several discrepancies among previous reports in terms of determining whether LTG has teratogenic effects in human fetuses of not.

The results of previous studies have indicated that oral intake of LTG in mice and rats at doses 1.2 times higher than the one used by humans (500 mg/kg) can be associated with no anomalies but could lead to preterm birth and decreased fetal weight.[14,27] However, Bastaki et al. and Padmanabhan et al. found that intraperitoneal administration of LTG, at doses above 75 mg/kg, could result in craniofacial anomalies such as cleft palate, exencephaly, and caudal.[15,29]

LTG can easily pass through the placenta and therefore affect fetal development and lead to anomalies.[30] The mechanism through which LTG causes anomalies remains to be adequately explained. It is believed that this drug decreases fetal folate levels that would be a main factor contributing to the development of congenital anomalies in humans.[29,31]

Essentially, because of these reasons, daily intake of 5 mg folic acid alongside AEDs, including LTG, in the first trimester helps to considerably decrease teratogenic effects.[32] Other evidence has indicated that the inclusion of serum levels methionine and free radicals produced by AEDs may be involved in the emergence of congenital anomalies in the fetus of pregnant women with epilepsy being treated with this drug.[6,7,15]

CONCLUSIONS

In conclusion, the use of LTG during pregnancy throughout organogenesis could have potential teratogenic effects and disturb fetal growth and development. In this study, ethanol as a substance with well-known teratogenic action was used for dissolving lamotrigine. Since the final concentration of injected ethanol for each mouse was less than 2%, less adverse effects of emerging anomalies

were experienced; so the obtained results should be interpreted with caution. According to these results, lamotrigine could be considered as a risk factor for the development of anomalies. The potentially decreased serum concentrations of folate and methionine could contribute to induce these anomalies. Therefore, more studies are needed to further investigate the effects of this medication and explain the relevant mechanisms.

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REFERENCES

1. Weinhold B. Environmental factors in birth defects: what we need to know. Environ Health Perspect 2009; 117(10): A440-7.
2. Lobo I, Zhaurova K. Birth defects: causes and statistics. Nature Education 2008; 1(1): 18.
3. Etemad L, Moshiri M, Moallem SA. Epilepsy drugs and effects on fetal development: Potential mechanisms. J Res Med Sci 2012; 17(9): 876-81.
4. Weston J, Bromley R, Jackson CF, et al. Monotherapy treatment of epilepsy in pregnancy: congenital malformation outcomes in the child. Cochrane Database Syst Rev 2016; 11: CD010224.
5. Morrow J, Russell A, Guthrie E, et al. Malformation risks of antiepileptic drugs in pregnancy: a prospective study from the UK Epilepsy and Pregnancy Register. J Neurol Neurosurg Psychiatry 2006; 77(2): 193-8.
6. Yerby MS, Kaplan P, Tran T. Risks and management of pregnancy in women with epilepsy. Cleve Clin J Med 2004; 71 Suppl 2: S25-37.
7. Cunnington M, Tennis P. Lamotrigine and the risk of malformations in pregnancy. Neurology 2005; 64(6): 955-60.
8. Holmes LB, Baldwin EJ, Smith CR, et al. Increased frequency of isolated cleft palate in infants exposed to lamotrigine during pregnancy. Neurology 2008; 70(22 Pt 2): 2152-8.
9. Richens A. Safety of lamotrigine. Epilepsia 1994; 35 Suppl 5: S37-40.
10. Kusumakar V, Yatham LN. Lamotrigine treatment of rapid cycling bipolar disorder. Am J Psychiatry 1997; 154(8): 1171-2.
11. Robakis TK, Holtzman J, Stemmle PG, et al. Lamotrigine and GABAA receptor modulators interact with menstrual cycle phase and oral contraceptives to regulate mood in women with bipolar disorder. J Affect Disord 2015; 175: 108-15.
12. https://pubchem.ncbi.nlm.nih.gov/compound/3878 NCfBIPCDCp.
13. Brunton L. Goodman and Gilman's The pharmacological basis of therapeutics. New York: Mcgraw-Hill Education; 2017.
14. Iqbal MM, Gundlapalli SP, Ryan WG, et al. Effects of antimanic mood-stabilizing drugs on fetuses, neonates, and nursing infants. South Med J 2001; 94(3): 304-22.
15. Bastaki S, Padmanabhan R, Abdulrazzaq Y, et al. Studies on the teratogenic effects of lamotrigine in mouse fetuses. Front Fetal Health 2001; 3(11-12): 295.
16. Marchi NS, Azoubel R, Tognola WA. Teratogenic effects of lamotrigine on rat fetal brain: a morphometric study. Arquivos de neuro-psiquiatria 2001; 59(2-b): 362-4.
17. Getova DP, Mihaylova AS. A study of the effects of lamotrigine on mice using two convulsive tests. Folia Med (Plovdiv) 2011; 53(2): 57-62.
18. Tehrani SP, Daryaafzoon M, Bakhtiarian A, et al. The effects of lamotrigine on the acquisition and expression of morphine-induced place preference in mice. PJBS 2009; 12(1): 33-9.
19. Banach M, Borowicz KK. Effects of chronic lamotrigine administration on maximal electroshock-induced seizures in mice. CNS Neurol Disord Drug Targets 2015; 14(7): 855-62.
20. Tan B. Guidelines on the care and use of animals for scientific purposes. National Advisory Committee for Laboratory Animal Research. 2004.
21. NRC. Guide for the care and use of laboratory animals. National Academy Press Committee to Revise the Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources. Washington DC; 1996.
22. Fazio A, Artesi C, Russo M, et al. A liquid chromatographic assay using a high-speed column for the determination of lamotrigine, a new antiepileptic drug, in human plasma. Ther Drug Monit 1992; 14(6): 509-12.
23. Deshmukh U, Adams J, Macklin EA, et al. Behavioral outcomes in children exposed prenatally to lamotrigine, valproate, or carbamazepine. Neurotoxicol Teratol 2016; 54: 5-14.
24. Rambeck B, Kurlemann G, Stodieck SR, et al. Concentrations of lamotrigine in a mother on lamotrigine treatment and her newborn child. Eur J Clin Pharmacol 1997; 51(6): 481-4.
25. Canavero S, Bonicalzi V. Drug therapy of trigeminal neuralgia. Expert Rev Neurother 2006; 6(3): 429-40.
26. Lampl C, Katsarava Z, Diener HC, et al. Lamotrigine

reduces migraine aura and migraine attacks in patients with migraine with aura. J Neurol Neurosurg Psychiatry 2005; 76(12): 1730-2.
27. Nahlik L. Lamotrigine (lamictal) when monoterapy just isn't enough. The University of Chicago Hospital 1996; 37: 5-6.
28. Wahid S, Khan RA, Feroz Z. Reduction in mortality and teratogenicity following simultaneous administration of folic acid and vitamin E with antiepileptic, antihypertensive and anti-allergic drugs. J Pharm Bioallied Sci 2014; 6(3): 185-91.
29. Padmanabhan R, Abdulrazzaq Y, Bastaki S, et al. Experimental studies on reproductive toxicologic effects of lamotrigine in mice. Birth Defects Res B Dev Reprod Toxicol 2003; 68(5): 428-38.
30. Ohman I, Vitols S, Tomson T. Lamotrigine in pregnancy: pharmacokinetics during delivery, in the neonate, and during lactation. Epilepsia 2000; 41(6): 709-13.
31. Tunbridge EM, Attenburrow MJ, Gardiner A, et al. Biochemical and genetic predictors and correlates of response to lamotrigine and folic acid in bipolar depression: Analysis of the CEQUEL clinical trial. Bipolar Disorders 2017; 19(6): 477-86.
32. Lagrange AH. Folic acid supplementation for women with epilepsy who might become pregnant. Nat Clin Pract Neurol 2009; 5(1): 16-7.

Оценка тератогенной активности противоэпилептического препарата ламотриджин на мышиных эмбрионах

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Введение: Приём антиэпилептических препаратов во время беременности может быть связан с повышенным риском тератогенности, а также врождённых аномалий. Тем не менее, существует много противоречивых мнений о том, может ли ламотриджин, как противоэпилептическое лекарственное средство, в значительной степени индуцировать пороки развития у новорожденных или нет. Таким образом, целью исследования является установление тератогенного воздействия ламотриджина на эмбрион мыши.

Материалы и методы: В настоящем исследовании 21 беременная мышь были распределены в 4 группы. Группы 1 и 2 (контрольные) были подвергнуты фиктивной обработке и 20% этанолу соответственно. Группы 3 и 4 (проходившие лечение) получали 25 и 75 мг / кг в течение 3 дней. Протокол лечения был выполнен в рамках 9-18 гестационного дня для всех групп. На 18-й день беременности 117 эмбрионов были удалены из маточных труб исследованных мышей и затем исследованы на наличие аномалий (позвоночника, конечностей и черепной коробки) с последующим измерением их роста и веса.

Результаты: Результаты показали, что в обработанных группах вес и рост были значительно снижены ($p < 0,01$) и установлено наличие различных аномалий. Кроме того, с увеличением дозы ламотриджина нарастали снижение веса, роста и тенденция к росту количества аномалий.

Заключение: Согласно полученным данным, ламотриджин можно рассматривать как фактор риска развития исследованных аномалий.