

Pathological findings in viscera of albino rat's fetuses of mother treated by lamotrigine

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Abstract

The aim of this study is to evaluate the effects of herapeutic doses and $\frac{1}{4}$ LD₅₀ of lamotrigine on the visceral structure of albino rat fetuses. This study was conducted over a period of 6 months extending from October 1, 2011 to February 1, 2012.

Sixty adult non-pregnant female albino rats and 30 male rats of the same strain, weighed 150-200 grams, were purchased from the Animal House of The Faculty of Medicine, Assiut University.

Sixty pregnant rats were used in this study and were classified into three groups:

a) Control Group (I): consisted of 20 pregnant females with normal saline administration. b) Study Group II: consisted of 20 pregnant females with therapeutic dose of lamotrigine oral administration of 5.4mg /d of lamotrigine, and (c) Study Group II: consisted of 20 pregnant females with $\frac{1}{4}$ LD₅₀ oral administrations of 32mg /d.

One hundred sixty eight fetuses (94 fetuses of GIIa and 74 fetuses of GIIb) and 134 fetuses from control group, were fixed in bouin's solution (aqueous saturated solution of picric acid 70% formalin 25% , glacial acetic acid 5%) for visceral examination. The study revealed that no internal visceral abnormalities were detected in fetuses of control and therapeutic doses of lamotrigine treated groups. Fetuses of the $\frac{1}{4}$ LD₅₀ treated group showed internal abnormalities in the heads cross sections only, while other levels sections showed no differences from control. Lamotrigine should not be regarded totally safe drug during pregnancy until its safety is established in a large scale randomized study with long term follow-up.

Key words: Lmotrigine, viscera, pathological findings, albino rats.

Introduction

The use of antiepileptic drugs (AEDs) in women with epilepsy (WWE) of child-bearing potential is a delicate balance between seizure control and adverse effects of AEDs, which are both potentially harmful to the developing fetus^(17,18).

The incidence of adverse effects is an important issue when prescribing antiepileptic drugs (AEDs), as some of the most effective medications for seizures are associated with a considerable degree of toxicity. Studies indicate that drug tolerance by individuals is a significant limiting factor in the treatment of seizure and drug retention rates are often determined by side-effect profiles^(3,4). Older AEDs may still be prescribed, owing to advantages that include lower cost, wide availability and long-term usage with known effects; but often exhibit greater toxicity than newer drugs. Newly developed agents tend to differ in terms of mechanisms of action and pharmacokinetic properties, and are often better tolerated than older drugs⁽⁷⁾.

Lamotrigine (LTG) is a newer anti-epileptic drug (AED), well-tolerated by children and adults with a wide-spectrum efficacy, in either monotherapy or polytherapy. It is approved for maintenance treatment of bipolar disorder without an indication for acute mania^(15,20). Moreover, it is also used in treatment-resistant unipolar depression as an augmentation drug and in post-traumatic stress disorder (Newer anticonvulsants)⁽¹⁵⁾. Effect of lamotrigine has been attributed to the inhibition of voltage-dependent sodium channels and a consequent inhibition of transmitter glutamate release⁽¹⁾. However, the mode of action of lamotrigine has been studied only in short-term animal experiments or in vitro⁽⁹⁾. Additionally, lamotrigine treatment reduced glucose metabolism in multiple regions of the cerebral cortex, basal ganglia, and thalamus in the patients with idiopathic generalized epilepsy⁽¹⁰⁾; which may produce pathological changes in the neocortex

and hippocampus⁽¹¹⁾. There are no published studies of pathological findings in fetuses of mother treated by lamotrigine.

Aim of the work

The aim of this study is to evaluate the effects of the therapeutic doses and $\frac{1}{4}$ LD₅₀ of lamotrigine on the visceral structure of albino rat fetuses.

Materials and Methods

This study is a randomized single blind control trial in which the visceral structure of albino rat fetuses of lamotrigine (AEDs) mothers was studied. Lamotrigine was used in two doses (Therapeutic and $\frac{1}{4}$ LD₅₀). This study was conducted over a period of 6 months extending from October 1, 2011 to February 1, 2012

Sixty female albino rats were used in this study, in association with thirty males of the same strain, which were used for mating.

A) Animals:

Sixty adult non pregnant female albino rats and 30 male rats of the same strain weighted (150-200 grams) purchased from animal house of faculty of medicine, Assiut University, the female rats were separated and housed for one week to be sure that they were non pregnant.

All over the period of experiment, the animals were housed in capacious cages with natural ventilation at room temperature (37°C). They received tap water and food (rodent pellets) ad libitum.

Two adult female albino rats were mated with one male rat of the same strain in the evening, a successful mating was known by vaginal redness and slight swelling on the following morning and regarded as Day 0 of gestation (GD0)⁽¹⁶⁾. The males were returned to their separate cages. Animals were classified randomly into three groups; each group contained 20 pregnant female rats.

A-Control groups: *Group I:* control group (n=20), received normal saline.

B-Treated groups:

Group IIa: received therapeutic dose of LTG (n=20).

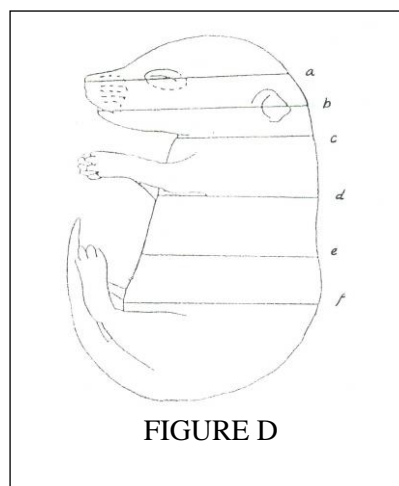
Group IIb: received $\frac{1}{4}$ LD₅₀ of LTG (n=20).

Animals in IIa group were given the therapeutic dose = 5.4 mg/day of lamotrigine ⁽²⁾, dissolved in 0.5 ml normal saline (0.25 ml twice/day), while animals in IIb group were given $\frac{1}{4}$ LD₅₀ = 32 mg/kg ^(active) of lamotrigine dissolved in 3.2 ml normal saline (1.6 ml twice/day) ⁽²⁾.

One hundred sixty eight fetuses (94 fetuses for GIIa and 74 fetuses for GIIb) and 134 fetuses from control group were fixed in bouin's solution (aqueous saturated solution of picric acid 70% formalin 25% , glacial acetic acid 5%) for visceral examination⁽⁶⁾. The internal organs of fetuses were studied by using the Wilson's razor blade technique. In order to do this, the whole fetuses were sectioned (3-5mm) in cranio-caudal direction (cross sections) as in Figure (D) and the slices sequentially examined under the binocular dissecting microscope for the presence of any gross abnormalities in the internal organs⁽¹⁹⁾.

Figure (D): A diagram showing the sites of the Wilson's section taken in 20-day old rat offspring. (Wilson, 1964).

- a- Section through the eyeball.
- b- Section through the ear and mouth.
- c- Section cranial to the forelimbs.
- d- Section caudal to the forelimbs.
- e- Section in the abdomen cranial to the umbilicus.
- f- Section in the abdomen caudal to the umbilicus.



Results

No internal visceral abnormalities were detected in fetuses of control and therapeutic doses of lamotrigine treated groups. Fetuses of the ¼ LD₅₀ treated groups showed internal abnormalities in the heads cross sections only, while the other levels sections showed no differences from control. (Figure 1 & 2).

Fetuses' number of ¼ LD₅₀ lamotrigine treated group was 74. They showed internal abnormalities in fetuses: (Table 1)

The brain showed areas of softening, indicating degeneration of the brain as in Figure (3).

Table 1: Distribution of pathological abnormalities among study groups

Pathological abnormalities (brain abnormalities)	Control Group (I) NO= 134 fetuses				Therapeutic study group (GIIa) NO= 94				¼ LD ₅₀ of lamotrigine study group (GIIb) NO=74			
	Present		Absent		Present		Absent		Present		Absent	
	No	%	No	%	No	%	No	%	No	%	No	%
Dilation of lateral ventricle	0	0.0	134	100.0	0	0.0	94	100.0	0	0.0	0	0.0
Shrinkage of brain	0	0.0	134	100.0	0	0.0	94	100.0	0	0.0	0	0.0
Degeneration changes (softening area)	0	0.0	134	100.0	0	0.0	94	100.0	74	100.0*	0	0.0
Delayed development of eye	0	0.0	134	100.0	0	0.0	94	100.0	0	0.0	0	0.0
Smoothness of upper palate	0	0.0	134	100.0	0	0.0	94	100.0	0	0.0	0	0.0
Absence of eyes	0	0.0	134	100.0	0	0.0	94	100.0	0	0.0	0	0.0
Others internal abnormalites	0	0.0	134	100.0	0	0.0	94	100.0	0	0.0	0	0.0

- Present in all fetuses but the areas involve are variable.
- P value = <0.0000 highly statistically significant.

Figure (1): Transverse section in the head of control group fetus, showing normal brain development and both eyes

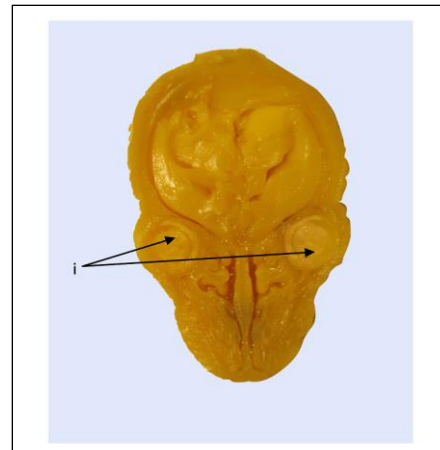


Figure (2): Transverse section in the head of control group fetus, showing complete development of palatal arch with its rugae

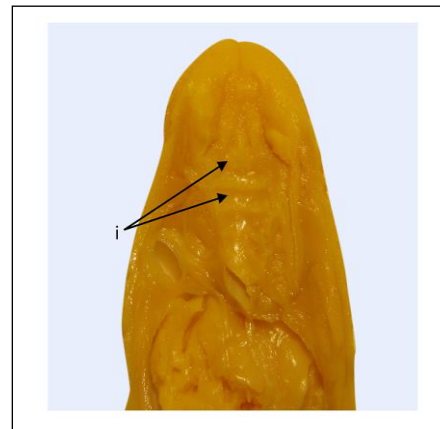
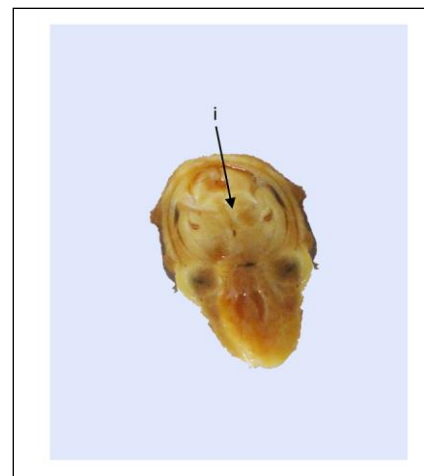


Figure (3): Transverse section in the head of 1/4 LD₅₀ lamotrigine group fetus, showing degeneration of brain.



Discussion

In our experimental study, all cross sections levels were normal in the therapeutic dose of lamotrigine study group, compared with control group, while findings were recorded only in offspring's brain of $\frac{1}{4}$ LD₅₀ lamotrigine treated rat; areas of softening and degeneration. These results in agreement with Marchi et al. (2001) who treated pregnant rats with four times the recommended human dose of LTG during the period of organogenesis and reported altered brain structure which included an increased volume and diameter of the cerebral structure, an increased density of the subcortical layer and ventricle dilatation. Manent et al. (2008) reported the presence of brain anomalies, which agreed with the current results, although these types of anomalies were different from that recorded in this study. Padmanabhan et al (2003) reported that administering lamotrigine as single dose of 50-200 mg/kg body weight can induce intrauterine growth retardation in mice, whereas multiple doses of 25, 50, 75 mg/kg body weight caused a dose dependent increase in embryonic resorption and craniofacial malformations. Mohanty et al (2011) and Elgndy et al (2016) showed an increase in the ventricular size in lamotrigine exposed rat fetus.

Conclusion

This animal study does not show significant gross morphometric effect of lamotrigine, some histological changes were observed in high dose administration. Therefore, LTG should not be regarded totally safe drug during pregnancy until its safety is established in a large scale randomized study with long term follow-up.

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التغيرات المرضية في أحشاء اجنة الفئران البيضاء للأمهات التي تعالج بالأمتروجين

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المخلص

تهدف الدراسة لتوضيح التغيرات المرضية الذي يحدثه الأمتروجين (أحد أدوية الصرع) لأجنة الفئران البيضاء بعد تعرضهم له داخل الرحم. استغرقت الدراسة ستة اشهر خلال الفترة من 1 أكتوبر 2011م حتى 1 فبراير 2012م. وأجريت على 60 فأراً بالغة غير حامل و30 ذكراً بالغاً من نفس السلالة لغرض التزاوج (معدل أوزانهم 150 - 200 جم) قُسمت الفأر الحوامل الى ثلاثة مجاميع: المجموعة الضابطة أعطي لها محلول ملحي، ومجموعة الدراسة الأولى أعطي لها الجرعة العلاجية من الأمتروجين (5.4 مجم/ يوم) والثانية أعطي لها الجرعة العلاجية الأخرى من العقار (32 مجم/يوم). ❖ مائة وثمانية وستون فأراً من مجموع فئران المجموعة الثانية أ (94) والمجموعة الثانية ب (74) و 134 من المجموعة الضابطة تم تثبيتهم في محلول البوان لغرض فحص الأحشاء الداخلية. تم الفحص النسيجي للشرائح تحت المجهر. لم تظهر العينات للمجموعة الضابطة والثانية أ من الجرعة العلاجية لعقار الأمتروجين تغييرات في الأحشاء الداخلية للفئران ، بينما ظهرت بعض التغيرات في كل الفئران من المجموعة الثانية ب وتركزت هذه التغيرات في الرأس فقط. بالرغم من ذلك لا يمكن أن نؤكد أن عقار الأمتروجين في جرعته العلاجية أ من حتى يتم اختباره على مجموعة عشوائية كبيرة ومتابعة طويلة.

الكلمات المفتاحية: الأمتروجين، أحشاء، نتائج مرضية، فئران بيضاء.