



ORIGINAL RESEARCH PAPER

Clinical Research

HISTOPATHOLOGICAL CHANGES IN DEVELOPING MICE KIDNEY TREATED WITH LAMOTRIGINE ON EARLY AND LATE PHASE OF GESTATION

KEY WORDS: Teratogenic, Lamotrigine, Microscopic, Kidney, Developing Mice.

Shweta Singh*

M.D. Anatomy. Hind Institute of Medical Sciences, Barabanki (U.P), India
*Corresponding Author

S.K. Pandey

M.S. Anatomy. Institute of Medical Sciences Banaras Hindu University, Varanasi (U.P).India.

ABSTRACT

INTRODUCTION-Lamotrigine (LTG) is an anti-epileptic drug (AED) and also used as a neuromodulator, in mood disorders.Metabolism is primarily achieved by competitive glucuronic acid conjugation. The principal product is an inactive lamotrigine-2-N-glucuronide conjugate, found predominantly in urine and to a lesser extent in feces. Recently, malformations have been reported in human foetuses, whose mothers were treated with LTG. However, it is not been possible to establish a recognizable pattern of malformations in the kidneys of human foetuses treated with LTG, therefore the lower animal has been used as experimental model for the present study.

OBJECTIVE- The objective of this study is to find out the microscopic changes in the kidney of the growing embryo of mice treated with LTG in both early and late phase of gestation.

MATERIAL AND METHODS –The pregnant mice were divided into two experimental group’s i.e. early and late, each with two subgroups i.e. control group (treated with intra peritoneal injection of normal saline on Day 4 and Day 9 of gestation) and treated group (treated with intraperitoneal injection of LTG ,150mg/kg body weight on 4th day and 9th Day of gestation. Fetuses were collected on day 18th of gestation and after fixation the kidneys were dissected out and processed for histological examination.

RESULT - The results of this study indicate that LTG administered intra peritoneally at high doses causes shrinkage of cortex and medulla and degenerated glomeruli, shrinkage of glomeruli and appearance of edematous spaces between the ducts.

CONCLUSION- In the present study histopathological changes were seen to be caused by LTG in the kidney of developing mice.

1.INTRODUCTION-

Lamotrigine (3,5-diamino-6-[2,3-dichlorophenyl]-1,2,4-triazine, C9H7Cl2N5) is an AED of the phenyltriazine class[1] and also used as a neuromodulator, in mood disorders [2].LTG crosses the placenta easily and rapidly, therefore maternal treatment leads to a considerable fetal exposure [3].Oral lamotrigine is rapidly absorbed with negligible first-pass metabolism. Peak plasma concentrations are reached in approximately 2–4 hours, and its half-life is approximately 25 hours Metabolism is primarily achieved by competitive glucuronic acid conjugation. The principal product is an inactive lamotrigine-2-N-glucuronide conjugate, found predominantly in urine and to a lesser extent in feces. The clearance of lamotrigine is reduced in the setting of renal insufficiency and hepatic disease. [1].Pennell et al. (2004)[4] observed an increasing magnitude of lamotrigine clearance during each trimester, reaching a peak of 330% of baseline clearance by week 32 of gestation. Plasma levels were noted to rapidly return to normal during the first few postpartum weeks. In newborns of mothers receiving lamotrigine, extensive placental transfer of drug has been found to occur, with umbilical cord concentrations approaching those of maternal serum. Drugs that have been found to be teratogenic in man have caused similar effects in animals [5, 6].The objective of this study is to find out the microscopic changes in the growing embryo of mice treated with LTG in early and late phase of gestation.

2. MATERIAL AND METHODS

The present study was conducted in the Teratology laboratory of the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi, after the approval of the Central Animal Ethical Committee. Thirty-two female Swiss albino mice of an average weight of 20-25 gm and about six weeks of age were used in this study. The animals were divided into four groups i.e. First Control Group (Gr-1), Early Treated Group (Gr-2), Second Control Group (Gr-3) and Late Treated Group (Gr-4).[7].

Female mice were transferred in the evening to the cages containing male mice of the same stock in the ratio of 3:1. The presence of vaginal plug on the following morning indicated the Zero Day of pregnancy. Each pregnant mice were weighed and divided into treated and control. Based on literature the dose was estimated to be 150mg per kg [8,9, and 10].The stock solution was made by dissolving 50mg strength of lamotrigine tablet in 5ml normal saline. Hence, approximately 0.4ml of stock solution would contain 4mg drug. Treated mice of each group received the drug intraperitoneally with the help of tuberculin syringe, whereas the

control group received equal volume of normal saline intraperitoneally.The pregnant mice were sacrificed with an overdose of ether anesthesia on day 18th of gestation.The fetuses were preserved in 10% formalin solution in a jar for fixation. After fixation the kidneys were dissected out and were further processed for paraffin embedding followed by H&E staining. The photomicrographs for histological studies were taken with the help of digital microscope (MOTIC 2000.1.13) and treated developing mice kidneys were compared with the control.

3. OBSERVATIONS and RESULTS-

The kidney of Group-2(early treated) showed shrinkage cortex and medullaand degeneration of glomeruli when compared with the corresponding control. In higher magnification, the renal cortex revealed destruction and shrinkage of glomeruli and appearance of edematous spaces in periglomerular area while the medulla showed destruction of ducts and their lining epithelium with appearance of edematous spaces between the ducts (Fig-1).These finding were further confirmed in oil immersion, The kidney of Gr-4 showed paucity of glomeruli and destruction of cortex and medulla when compared with its corresponding control. In higher magnification, decreased thickness of cortex was well appreciated. There was increased destruction and degeneration of the glomeruli and periglomerular ducts in the renal cortex, while the medulla showed destruction of the normal architecture, when compared with the corresponding control (Fig-2).

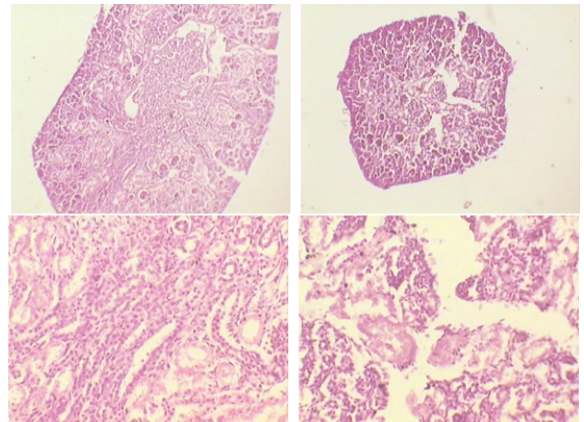


FIG-1. Microscopic Structure Of Growing Kidney Of First Experimental Group

Showing shrinkage of cortex and medulla, degenerated glomeruli and appearance of edematous spaces in the medulla of kidney of treated fetuses (B) when compared with control (A) when viewed in 100x. In higher magnification (400x), destruction of ducts & their lining epithelium in medullary region with edematous spaces between the ducts can be clearly seen in the treated group (D) when compared with the normal appearance seen in the control group ©.

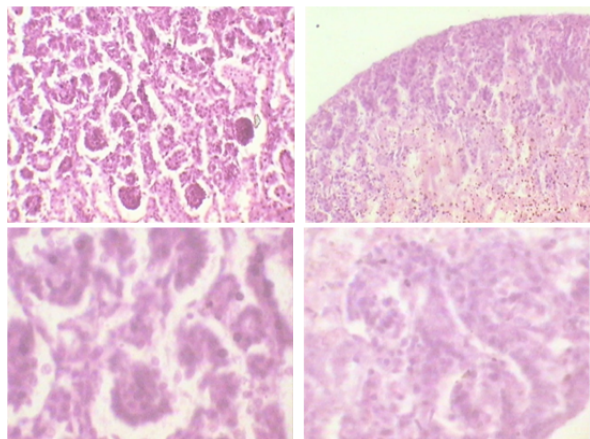


FIG-2 Microscopic Structure Of Growing Kidney Of Second Experimental Group: Showing decrease in thickness of the cortex and increased destruction and degeneration of the glomeruli and periglomerular ducts in renal cortex of treated fetus(B) when compared with control(A) in 100x magnification. In oil immersion degenerative appearance of renal glomeruli and reduction in periglomerular space and increase in cellular density around periglomerular region was noted in the treated fetuses (D) when compared with the normal appearance seen in control group ©.

DISCUSSION. In newborns of mothers receiving lamotrigine, extensive placental transfer of drug has been found to occur, with umbilical cord concentrations approaching those of maternal serum. Nursing infants demonstrate plasma lamotrigine concentrations approximately 23%–50% of maternal levels. (Ohman et al, 2000). Thus the growing embryo are exposed to very high level of this drug in utero when the pregnant mother receives this drug and this might lead to the various noxious effects on the developing fetus. In Reproductive toxicity studies, the test substance is commonly administered throughout the period of organogenesis. This procedure may have the advantage of covering the entire susceptible period, but the disadvantage is that continuous dosing might activate maternal adaptive mechanisms, mask certain embryonic responses that are developmental stage specific and produce 'misleading results'. It was further described that rodents differ significantly from humans in their metabolism and ability to handle drugs and for this reason teratological studies on anti-epileptic drug (AED) most commonly employ doses several times higher than recommended human doses[11,12]. Few studies have been carried out to ascertain the effects of lamotrigine on developing fetal kidney[13]. As described earlier in the text many changes were found to occur in the developing kidney of treated mice when exposed to LTG in utero.

CONCLUSION-

Kidneys of developing mice who were exposed to in utero lamotrigine has shown various noxious effects on histopathological examination. Teratological study are undertaken to anticipate risk before they materialize. Though the findings on animals cannot be directly extrapolated on humans, discretion should be applied to weigh the benefits of treatments against the risk of developing teratological changes.

REFERENCES

1. GlaxoSmithKline: Lamictal (lamotrigine) prescribing information. Research Triangle Park, NC, GlaxoSmithKline, May 2007.
2. Bowden CL, Calabrese JR, Sachs G, et al: A placebo-controlled 18-month trial of lamotrigine and lithium maintenance treatment in recently manic or hypomanic

3. patients with bipolar I disorder. Arch Gen Psychiatry 60:392–400, 2003.
4. Ohman I, Vitols S, Tomson T: Lamotrigine in pregnancy: pharmacokinetics during delivery, in the neonate, and during lactation. Epilepsia 41:709–713, 2000
5. Pennell PB, Newport DJ, Stowe ZN, et al: The impact of pregnancy and childbirth on the metabolism of lamotrigine. Neurology 62:292–295, 2004.
6. Koren G, Pastuszak A, Ito S. Drugs in pregnancy. N Engl J Med 338:1128–37, 1998.
7. Marchi NS, Azoubel R, Tognola WA. Teratogenic effects of lamotrigine on rat fetal brain: a morphometric study. ArqNeuropsiquiatr 59:362–4, 2001
8. Singh S and Pandey S.K. Adverse macroscopic changes in developing mice treated with Lamotrigine on early and late phase of gestation. International Journal Of Current Advanced Research Vol.7, issue, 2(G), pp. 10104-10107, February, 2018.
9. Padmanabhan R, Abdulrazzaq YM, Bastaki SM, Shafiullah M, Chandranath SI. Experimental studies on reproductive toxicologic effects of lamotrigine in mice. Birth Defects Res B Dev Reprod Toxicol 68:428–38, 2003.
10. Iqbal MM, Gundlapalli SP, Ryan WG, Ryals T, Passman TE. Effect of antimanic mood-stabilizing drugs on fetuses, neonates, and nursing infants. South Med J 94:304–22, 2001
11. Rahmani F, Delaram M, Forouzandeh N: The teratogenic effects of Lamotrigine on mouse fetus. Journal of Reproduction and Infertility, 7(1):45–52, 2006.
12. Wilson JG. Environment and birth defects. New York: Academic Press. 1973
13. Wilson JG. Current status of teratology. The handbook of teratology. 2.Ed. New York: Plenum Press 47–74, 1979.
14. Cunningham M., Ferber S. Effect of Dose on the Frequency of Major Birth Defects Following Fetal Exposure to Lamotrigine Monotherapy in an International Observational Study. Epilepsia, 48(6): 1041–1222, 2007.