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Possible effects of lamotrigine on liver of wister rats exposed to chemoconvulsion and Chronic Restraint model

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Abstract: The present study is designed to investigate the possible hepatotoxic effects of the anti-epileptic drug "lamotrigine, LTG" in adult male wister rats after picrotoxin –induced convulsions and exposure for 21 days to chronic restraint model. This was done by a trial to find out alterations in the activities of liver enzymes and some antioxidants in this model of co-morbidity. They were treated by gastric gavage with LTG [20mg/kg body wt.] for 21days. Then rats were anesthetized and dissected to remove liver and to collect blood. Selected liver enzymes [AST, ALT] and some anti-oxidant enzymes were assayed. The results indicated that the drug significantly increased the activities of glutathione peroxidase and catalase enzymes in hepatic homogenates, while it significantly decreased the level of the lipid peroxidation expressed as thiobarbituric acid-reactive substance (TBARS) in these homogenates. However, there was an elevation of tested liver enzymes ALT & AST at the end of 21 days. This revealed the occurrence of possible hepatocellular damage. The present study recommends a regular liver function and drug monitoring during the therapeutic use of this drug in epilepsy-stress co-morbidity.

Keywords: Lamotrigine, Picrotoxin, Chronic Restraint Model, Liver, Rats

1. Introduction

Process of epileptogenesis and long-term use of certain antiepileptic drugs have been shown to cause increase in some plasma concentration biomarkers as lipoproteins [1], plasma concentration of homocysteine (Hcy) with an increase in reactive oxygen species [2]. A number of experimental and clinical reports suggest the involvement of oxidative stress in pathophysiology of epilepsy is apparently related increased free radicals result in membrane lipid peroxidation of various body organs as brain, liver, heart etc. and decreased glutathione concentrations in the epileptic focus of the brain [3]. Old generation of anti-epileptic drugs may impair the endogenous antioxidative ability and hence lead to oxygen-dependent tissue injury [4].

The excess production in generation of mitochondrial ROS due to tissue toxicity initiates a damaging circle by activating stress-sensitive pathways such as NF-κB, p38 MAPK, Jak/STAT, PKC and pro-inflammatory cytokines that contribute to many complications [5 &6]. Furthermore, there is an increase in the formation of oxidant peroxynitrite that plays an important function in experimental and clinical organ damage.

There is strong evidence that any drug that can reduce seizure activity would also lower TBARS (Thiobarbituric acid reactive substances) levels, as a marker of lipid peroxidation. This Reduction would also give an indication of the additional neuroprotective and/or antioxidant properties of the drug [2].

Lamotrigine (Lamictal) is used as adjunctive therapy or monotherapy in adults with partial seizures with or without secondary generalization. It acts also on voltage-sensitive sodium channels and stabilizes neural membranes with inhibition of the release of excitatory neural transmitter such as glutamate. [4].

Many clinical reports from prescribing anti-epileptic drugs demonstrated that major antiepileptic drugs can cause hepatotoxicity. Hepatic reactions to LTG ranged from transient elevation of hepatic enzymes without clinical

signs or symptoms of hepatic dysfunctions to fatal hepatotoxicity (5, 6). In the light of potential toxicity, clinicians prescribing LTG as a single therapy or polytherapy must be alert to the possibility of serious hepatic reactions particularly in case of polytherapy with enzyme inducing agents, each of which may contribute to the overall risk of hepatic dysfunction.

The relative role of LTG in elevation of liver enzymes as a whole is difficult to assess on the basis of case reports alone and it needs to be supported by animal studies, because of various factors interfering the results in human cases (7).

The present study is performed to evaluate its potential hepatotoxicity using an anti-convulsant dose determined from a pilot study, by analyzing ALT & AST liver enzymes and some anti-oxidant markers in liver homogenates of male wister rats exposed to picrotoxin-induced convulsions and chronic restraint model for 21 days as a co-morbid state.

2. Materials and Methods

2.1. Drugs and Chemicals

Lamotrigine (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in a mixture of 0.5% carboxymethylcellulose, 0.4% Tween 80, 0.9% benzylic acid and saline. All other chemicals were obtained from Sigma Chemical Co (St Louis, MO).

2.2. Animals

Male Wister rats (200–250 g) were divided into three groups with 12 rats each. They were housed in cages with a natural light-dark cycle and fed on a standard pellet diet and water ad libitum

Chemoconvulsion model in both groups (2&3):

Exposure of tested groups to picrotoxin (PTX)-induced convulsions then to chronic restraint stress procedure:

Groups 2 &3 were given a single subcutaneous dose (3.5 mg/kg body weight) of PTX following administration of the single dose of the test drug (treated groups either by vehicles [group 2] or by lamotrigine [group 3]).

PTX-injected groups (2&3) were injected after a suitable latency corresponding to the time expected to reach a peak effect following administration of the respective test drug.

The latency of lamotrigine was estimated to be 2 hour after its gastric gavage administration, as determined by the pilot study. Immediately after administration of picrotoxin, the animal was observed for 30 minutes. The onset of convulsive behavior as well as nature and severity of convulsions were carefully recorded using the scoring system 1-7: Group (1) = normal movement and contractility in the scoring process. Group (2)= mean scoring process = 5 =tonic forelimb convulsions followed by clonus. Group (3) = mean scoring process = 1 =hyper-locomotion or piloerection (Erection of the skin hair) [Please refer to Appendix A of convulsive score used in the present study]

Chronic Restraint model done on both control (group (1) and in groups (2&3) after induction of chemocunvulsion

At the end of the PTX study for each group, rats were returned to their cages to continue with the chronic restraint stress study. All rats of the different groups (see below) were placed in a wire mesh restrainer 6 hours daily for 21 days. At the end of the restraint period, the rats were moved to their cages. All these procedures were repeated daily for 21 days.

2.3. Animal Grouping

1. Control group did not receive picrotoxin or lamotrigine.
2. Control group received picrotoxin, as a model of chemoconvulsion, but did not receive lamotrigine, only its vehicles by gastric gavage.
3. Lamotrigine-treated group: received lamotrigine dissolved in a mixture of 0.5% carboxymethylcellulose, 0.4% Tween 80, 0.9% benzylic acid and saline as a single dose of 20 mg/kg body weight by gastric gavage. Then they received PTX to induce chemoconvulsion that was almost absence in all rats of group (3) due to the anti-convulsant effect of lamotrigine.

2.4. N.B.

The dose of LTG was determined after doing a picrotoxin-induced model of chemoconvulsion and was found to be effective anti-convulsant dose.

The dose of LTG that was lesser than the used dose was also anti-convulsant but without affection of liver enzymes or the anti-oxidant markers. The results were comparable to the control group. So in the present study, we concentrate on the first dose that start to induce hepatic anti-oxidant effect, but causes an elevation in ALT & AST enzymes of the liver. This would recommend further biochemical and histopathological studies using the higher doses.

2.5. Measurements

1. Serum levels of alanine and aspartate transaminases (ALT &AST)

They were measured using biochemistry automatic analyzer (Hitachi 7600)

2. Measurement of the level of TBARS in liver homogenates of tested rats as a marker of lipid peroxidation [8]

Parts of liver homogenates of each rat in each group were rinsed with cold 0.14 M NaCl, and part of it was homogenized in 25% ice cold 50 mM Tris-HCl buffer, pH 7.4. One hundred and fifty microliters of the tissue supernatant of samples was diluted to 500 µL with deionized water. A total of 250 µL of 1.34% thiobarbituric acid was added to all the tubes, followed by the addition of an equal volume of 40% trichloroacetic acid.

The mixture was shaken and incubated for 30 minutes in a boiling water bath. Tubes were allowed to cool to room temperature and the absorbance was read at 532 nm using

zero concentration as blank.

3. Effect of lamotrigine on the activities of liver homogenates of catalase and glutathione peroxidase of tested rats

a) Catalase enzyme activity [9]

Catalase activity in the liver homogenates was assayed by spectrophotometer using dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in a 1:3 ratio). Intensity was measured at 620 nm, and the amount of hydrogen peroxide hydrolyzed was calculated for the catalase activity.

b) Glutathione peroxidase enzyme activity [10]

Glutathione peroxidase activity in liver homogenates was measured. Activity was expressed based on inhibition of glutathione.

2.6. Protein Determination [11]

The total protein content of liver homogenates was determined. The aim was to express the TBARS concentration as nmol/mg tissue protein, and catalase and glutathione peroxidase enzyme activity as Unit/mg tissue protein.

2.7. Ethics

All procedures were in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, as well as the guidelines of the Animal Welfare Act.

2.8. Data Analysis

Results are expressed as mean±SD [Standard Deviation]. Statistical analysis was performed by analysis of variance followed by Tukey's post hoc using GraphPad Prism version 3.00 for Windows 97 (Graph Pad Software, San Diego, CA, U.S.A.). Differences with $p < 0.05$ were considered to be statistically significant.

3. Results

1. Serum levels of aspartate & alanine amine transferase (AST, ALT) in U/L

Table (1). *Showed significant ($p<0.05$) increase in both serum AST & ALT in lamotrigine-treated rats group compared with control group.*

mean±SD of serum enzyme levels			
	Control	**Chronic restraint non-treated**	**Chronic restraint lamotrigine-treated group**
ALT	41.75±4.42	50.5±4.5	731.43±5.4*
AST	41.95±3.19	55.9±3.2	641.12±4.8*

* $p<0.05$= significant increase in liver enzymes in chronic restraint lamotrigine-treated group compared to both groups 1 &2.

2. Effect of lamotrigine on the level of TBARS in liver homogenates of tested rats as a marker of lipid peroxidation

Figure (1) showed significant ($p<0.05$)reduction of lipid peroxidation, expressed by TBARS in nmol/mg tissue protein of liver homogenates of rats of LTG-treated group (3) compared to group (2) that was exposed to PTX without LTG treatment. However, its level was significantly increased ($p<0.05$) in group (2) compared to control group (1).

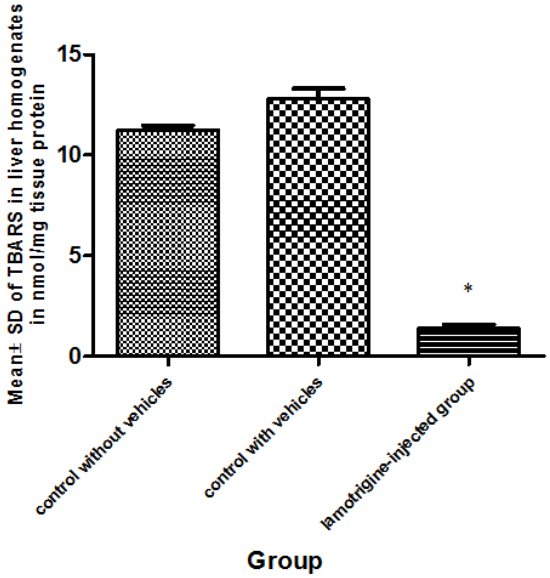

Figure (1). *Lamotrigine administration to group 3 significantly (* $p < 0.05$) lowered TBARS level compared with the control groups exposed to chronic restraint model (groups 1 &2).*

In a pilot study, done before the present study, control group [not exposed to chronic restraint model nor vehicles or lamotrigine injections], TBARS level was approximately close to the value reported with group (3) treated with lamotrigine.

3. Effect of lamotrigine on the activities of liver homogenates catalase and glutathione peroxidase

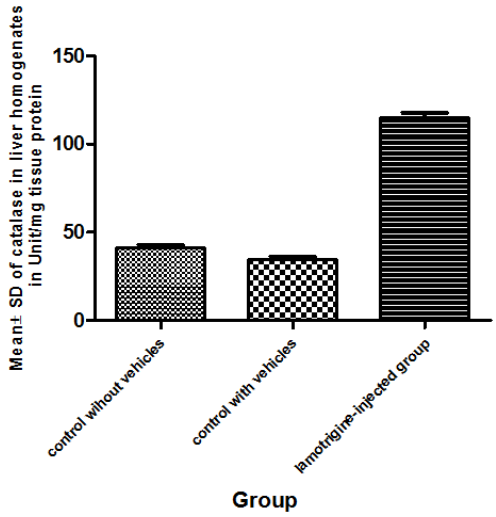

Figure (2). *Changes in the activity of liver homogenates catalase in different tested groups of rats.*

Figure (2) showed:

- Significant decrease in the activities of the

antioxidant enzyme in groups (1 & 2) exposed to chronic restraint model.

- While the lamotrigine- treated Group (3) showed that the activities of the enzyme as an antioxidant was significantly (* $p < 0.05$) increased compared to control groups (1 &2).

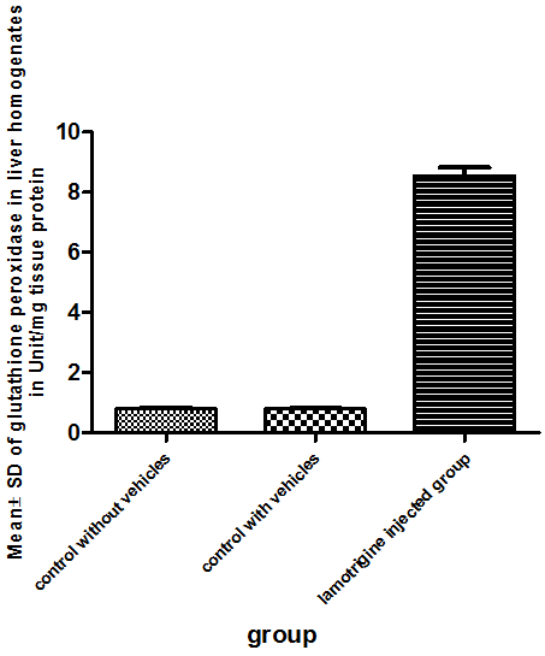

Figure (3). *Changes in the activity of glutathione peroxidase enzyme in the tested liver homogenates of different tested groups of rats.*

Figure (3): significant (* $p < 0.05$) increase in activity of the measured enzyme versus Groups 1, 2

In a pilot study, done before the present study, control group [not exposed to chronic restraint model nor vehicles or lamotrigine injections], activities of both catalase and glutathione peroxidase enzymes were approximately close to the values reported with group (3) treated with lamotrigine.

N.B. Groups (2&3) are exposed to convulsions by picrotoxin as reported in the methodology.

4. Discussion

The results of the present study, done on liver homogenates in rats exposed to chronic restraint model, revealed that gastric gavage administration of lamotrigine at a dose of 20mg/kg/day for 21 days as an anti-epileptic drug, resulted in a significant increase in both ALT & AST liver enzymes, a decrease in TBARS content with an increase in the activities of both catalase and glutathione reductase enzymes.

A clinical study, that was done on three children suffering from seizures and were treated with lamotrigine, showed that one child suffers from severe hepatic failure due to aggressive therapy. However, after discontinuation of the drug, his liver function and blood hepatic enzymes became normal. On the other hand, some other studies reported that the lower dose of LTG caused a mild elevation of liver enzymes but no liver cell damage (12,13).

5. Conclusion

In conclusion, the experimental depression is associated with elevated oxidative stress although treatment with lamotrigine has most protective effects on the oxidative stress within three medicines [24].

Blockade of presynaptic release of glutamate by lamotrigine treatment yielded protective effects on the spinal cord ultrastructure even when administered after the spinal cord injury (SCI); it also prevented oxidative stress when it was administered before or during the SCI [25].

The effect of carbamazepine and lamotrigine was assessed on cognitive function and oxidative stress in brain during chemically induced epileptogenesis in rats. Epileptogenesis was induced by administration of pentylenetetrazole (30 mg/kg, s.c.) on alternate days (three times/week) for 9-11 weeks or until stage 4 of seizure score was achieved. The neurobehavioral parameters used for cognitive assessment were step-down latency in continuous avoidance apparatus and transfer latency in elevated plus maze test paradigm. Carbamazepine and lamotrigine were administered intraperitoneally in doses of 60 mg/kg and 25 mg/kg, respectively, according to the groups, once a day for 11 weeks. Oxidative stress was assessed in isolated homogenized whole brain samples and estimated for the levels of malondialdehyde, reduced glutathione, catalase and superoxide dismutase. The results showed that lamotrigine did not produce any change in cognitive function, while carbamazepine produced cognitive dysfunction. Cognitive decline seen in the carbamazepine-treated pentylenetetrazole-kindled group was also associated with increased oxidative stress. Lamotrigine treatment had no effect on oxidative stress parameters alone, while it significantly decreased oxidative stress in the pentylenetetrazole-kindled group as compared to the pentylenetetrazole-kindled carbamazepine-treated group [13,14].

Although the anti-oxidant effect of LTG in an experimental model of spinal cord injury (15), however, hepatic reactions to LTG ranged from transient elevation of hepatic enzymes without clinical signs or symptoms of hepatic dysfunctions to fatal hepatotoxicity . In the light of potential toxicity, clinicians prescribing LTG as a single therapy or polytherapy must have attention to the possibility of occurance of an autoimmune LTG-induced hepatitis up to reaching many serious hepatic reactions particularly in case of polytherapy with enzyme inducing drugs as carbamazepine, keeping in mind that many anti-epileptic drugs, possess an apparent anti-oxidant effect on different body organs e.g. brain and liver, however they may contribute to a great risk of hepatic dysfunction (16). .

We need to do further investigations in experimental chemoconvulsive studies to prove the beneficial drug interactions in cases of epilepsy and stress comorbidity

with and without hepatic disorders. In most developing countries, financial markets have grown rapidly during the last two decades due to several reasons such as the information technology revolution, deregulation and globalization. Integration among the countries has grown during this period all over the world. This is mainly due the direct relationship with the economy, given the important role of the financial markets in the real economic activities (17). We hope to reach an optimal financial support for scientific research in all arab countries such as Egypt, Kuwait and Jordan where epilepsy is a common neurological disease in different ages.

In conclusion, the management of epilepsy-stress co-morbidity by LTG could be associated with possible beneficial anti-oxidant actions on different body organs in addition to its anti-convulsant effect in mice exposed to a chemoconvulsive model. The later effect could be partially related to a decrease in levels of some oxidative stress markers as TBARS with an increase in anti-oxidant enzymes e.g. catalase and glutathione peroxidase enzymes as proved in the present study. Clinically, a strong recommendation of a careful monitoring of liver function tests and LTG drug monitoring should be done while treating stressed epileptic patients with LTG, with an avoidance of the prescription of large doses as far as possible, especially in people who live in developing countries with the presence of a large percentage of them suffering from endemic hepatitis.

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Disclosure

The author reports no conflicts of interest in this work.

Appendix

The severity of convulsions was carefully recorded using the scoring system 1-7 as follows: hyper-locomotion or piloerection (Erection of the skin hair) –1; stunning (immobile) or catatonic posture (assuming a fixed posture and inability to move) –2; clonic body tremors (a series of involuntary muscular contractions due to sudden stretching of the muscle) –3; prolonged clonic tremors – 4; tonic forelimb convulsions followed by clonus –5; repetitive tonic (prolonged muscular contraction) forelimb convulsions followed by clonus –6; tonic extension of both forelimbs and hindlimbs followed by clonus – 7. A mean cumulative score was calculated and wrote beside each group.

References

[1] Nikolaos T, Stylianos G, Chryssoula N, Irini P, Christos M, Dimitrios T, Konstantinos P, Antonis T. The effect of long-term antiepileptic treatment on serum cholesterol (TC, HDL, LDL) and triglyceride levels in adult epileptic patients on monotherapy. Med Sci Monit. 2004;10:MT50-52.

[2] Attilakos A, Papakonstantinou E, Schulpis K, Voudris K, Katsarou E, Mastroyianni S, Garoufi A. Early effect of sodium valproate and carbamazepine monotherapy on homocysteine metabolism in children with epilepsy. Epilepsy Res. 2006;71: 229-232.

[3] Sudha K, Rao AV, Rao A. Oxidative stress and antioxidants in epilepsy. Clin Chim Acta. 2001;303:19-24.

[4] Fabio F, Annarosa L, Daniela C, Federica L and Bijan S. Hyperglycemia activates p53 and p53-regulated genes leading to myocyte cell death. Diabetes, 2001; 50: 2363-2375.

[5] Ziegler D, Rathmann W, Dickhaus T, Meisinger C and Mielck A and KORA Study Group. Neuropathic pain in diabetes,prediabetes and normal glucose tolerance: the MONICA/KORA Augsburg Surveys S2 and S3. Pain Med. 2009; 10: 393-400

[6] William J. Curry, M, And Kulling D, Milton S.Newer Antiepileptic Drugs: , Lamotrigine, Felbamate, Topiramate and Fosphenytoin. 1998; 57(3):513-520.

[7] Overstreet, K., Costanza, C., Behling, C.,Hassanin, T. and Masliah, E. (2002). Fatal progressive hepatic necrosis associated with lamotrigine treatment: A case report and literature review. Dig. Dis.Sci. 47, 1921-1925.

[8] Gutteridge J and Quinlan G (1983). Malondialdhyde formation from lipid peroxides in the thiobarbituric acid test: the role of lipid radicals, iron salt and metal chelators. J Appl Biochem. 1983; 5(4–5):293–299

[9] Sinha KA. Colorimetric assay of catalase. Ann Biochem. 1972; 47: 389–394.

[10] Rotruck J, Pope A, Ganther H, Swanson A, Hafeman D, Hoekstra W. Selenium. Biochemical role as a component of Glutathione peroxidase. Science. 1973; 79:588–590.

[11] Bradford M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal. Biochem. 1976; 72:248-254

[12] Meldrum, B.S. (1994). Lamotrigine a novel approach. Seizure 3 suppl A, 41-45.

[13] Nanxin Li, Xiaolu He, Xiaoli Qi, Yu Zhang and Shuchang He. The mood stabilizer lamotrigine produces antidepressant

[14] Eren I, Nazıroğlu M and Demirdaş A. Protective Effects of Lamotrigine, Aripiprazole and Escitalopram on Depression-induced Oxidative Stress in Rat Brain. Neurochem. Res. 2007; 32(7): 1188-1195

[15] Tufan K, Oztanir N, Ofluoglu E, Ozogul C, Uzum N, Dursun A, Pasaoglu H, Pasaoglu A. Ultrastructure protection and attenuation of lipid peroxidation after blockade of presynaptic release of glutamate by lamotrigine in experimental spinal cord injury. Neurosurg. Focus. 2008;25(5):E6

[16] Overstreet K, Costanza C, Behling C,Hassanin T and Masliah E. Fatal progressive hepatic necrosis associated with lamotrigine treatment: A case report and literature review. Dig. Dis.Sci.2002; 47, 1921-1925.

[17] Mohamad H. Atyeh, Wael Al-Rashed. Testing the Existence of Integration; Kuwait and Jordan Financial Markets. International Journal of Economics, Finance and Management Sciences.2013; 1(2), pp. 89-94. doi: 10.11648/j.ijefm.20130102.14