Possible Anti-oxidant Effect of Lamotrigine in Nucleus Accumbens of Mice Exposed to Picrotoxin

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Abstract
The present study was performed to investigate whether or not lamotrigine (an anti-epileptic drug by sodium-channel blockade and also increases gamma-aminobutyric acid [GABA]) could increase some anti-oxidant markers in the nucleus accumbens of mice exposed to picrotoxin as a model simulating convulsions in human. The results indicated that the anti-epileptic drug significantly increased the activities of glutathione peroxidase and catalase enzymes in nucleus accumbens homogenates as well as the contents of superoxide dismutase enzyme in red blood cells (RBCs) lysates in this model of convulsion. In addition the tested drug significantly decreased the level of the lipid peroxidation expressed as thiobarbituric acid-reactive substance (TBARS) in nucleus accumbens homogenates. So, the present study showed that the tested antiepileptic drug could potentiate its anticonvulsant activity via a possible anti-oxidant activity.

Keywords: Lamotrigine; Picrotoxin; Antioxidant; Mice

Introduction
Oxidative stress is a common mechanism in the causation of many diseases including organ damage [1]. The increase in production of Reactive Oxygen Species (ROS) is found to be contributed to nerve injury [1,2]. In normal neuron, ROS production is tightly regulated. The free radical superoxide is generated by mitochondrial electron transfer chain when nicotinamide adenine dinucleotide (NADH) is oxidised to NAD*. Superoxide produced in various diseases rapidly combines with NO and the formed peroxynitrite causes protein nitration or nitrosylation, lipid peroxidation, DNA damage and cell death and has direct toxic effects on the nerve tissue leading to neuronal damage, neuropathic pain and convulsions [3,4]. Mitochondria in neuron is sensitive to oxidative damage-which results impaired energy regulatory function that leads to loss of neuronal function and the development of PDN [1]. Moreover, excess generation of mitochondrial ROS due to neuronal toxicity initiates a vicious circle by activating stress-sensitive pathways such as NF-kB, p38 MAPK, Jak/STAT, PKC and pro-inflammatory cytokines that contribute to many complications [5,6]. In addition, there is an increase in the formation of potent oxidant peroxynitrite, which is formed by the combination of superoxide anion radical with nitric oxide and the formed peroxynitrite has been documented to play a key role in experimental and clinical neuropathy. Peroxynitrite causes protein nitration and nitrosylation of biomolecules including proteins, lipids, DNA and has a direct toxic effect on neurons leading to complications in the nervous systems [4,7].

There is strong evidence supporting the correlation between oxidative stress and epilepsy. Free radicals are involved in the pathogenesis of various diseases including epilepsy [8]. Chemo convulsions, e.g. picrotoxin-induced convulsions, are followed by the generation of free radicals that cause lipid peroxidation, which may subsequently cause neurodegeneration observed in certain types of human epilepsy [9]. Therefore, a drug that reduces seizure activity should also lower TBARS (Thiobarbituric acid reactive substances) levels. Reduction of TBARS levels compared to those by chemoconvulsants alone would also give an indication of the additional neuroprotective and/or antioxidant properties of the drug [10].

Lamotrigine (Lamictal) is included in the phenytoin class. It is used as adjunctive therapy or monotherapy in adults with partial seizures with or without secondary generalization. Lamotrigine has been shown to act at voltage-sensitive sodium channels, stabilizing neural membranes and inhibiting the release of excitatory neural transmitter [11].

The nucleus accumbens (NAcc) (The pleasure center) is the studied brain area of tested mice. It is formed of a collection of neurons and considers being the main part of the ventral striatum. It is thought to play an important role in reward, pleasure, laughter, addiction, aggression, fear. However, no detectable experimental studies pointed to its possible role in pathogenesis and treatment of epilepsy [12].

The present study is performed to investigate whether or not lamotrigine, as an anti-epileptic drug, could increase or decrease some anti-oxidant markers in the nucleus accumbens (whose main neurotransmitter of its neurons is GABA) of mice exposed to picrotoxin as a model simulating convulsions in human.

Materials and Methods

Drugs and chemicals
Picrotoxin (PTX; Sigma-Aldrich Co, St Louis, MO), 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 and for measurement of superoxide dismutase enzyme in red blood cells (RBCs) lysates in this model of convulsion. In addition the tested drug significantly decreased the level of the lipid peroxidation expressed as thiobarbituric acid-reactive substance (TBARS) in nucleus accumbens homogenates. So, the present study showed that the tested antiepileptic drug could potentiate its anticonvulsant activity via a possible anti-oxidant activity.

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Animals

Albino mice (20–25 g) were divided into three groups with 12 mice each. They were housed in cages with a natural light-dark cycle and fed on a standard pellet diet and water ad libitum.

A) Picrotoxin-induced convulsions

All mice were given a single subcutaneous dose (3.5 mg/kg body weight) of PTX either in the absence of any treatment (control group) or following administration of a single dose of the test drug (treated groups either by vehicles [group 2] or by lamotrigine [group 3]). Accordingly, the study comprised the following three groups:

1. Control group did not receive lamotrigine.
2. Control group did not receive lamotrigine, only its vehicles.
3. Lamotrigine-treated group: received lamotrigine dissolved in a mixture of 0.5% carboxymethylcellulose, 0.4% Tween 80, 0.9% benzylic acid and saline [13] as a single dose of 20 mg/kg body weight intraperitoneal [14].

In the treated group, PTX was 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 1 hour after its intraperitoneal administration, as determined by the pilot study.

Immediately after administration of PTX, the animal was observed for 30 minutes. The onset of convulsive behavior as well as the nature and severity of convulsions were carefully recorded using the scoring system 1–7 as follows: hyperlocomotion 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; and tonic extension of both forelimbs and hindlimbs followed by clonus, 7; a mean cumulative score was calculated for lamotrigine-treated group for comparisons and statistical analysis.

B) Exposure of tested groups to chronic restraint stress procedure followed by the antioxidant tests:

At the end of the PTX study for each group, the animals were returned to their cages to continue with the chronic restraint stress study.

Each mouse of the respective group was placed in a wire mesh restrainer 6 hours daily for 21 days. At the end of the restraint period, the mice were moved to their cages.

For the purpose of the antioxidant tests, the following three groups were classified as followed:

1. Control group did not receive lamotrigine.
2. Control group did not receive lamotrigine, only its vehicles.
3. Lamotrigine-treated group: received lamotrigine dissolved in a mixture of 0.5% carboxymethylcellulose, 0.4% Tween 80, 0.9% benzylic acid and saline [13] as a single dose of 20 mg/kg body weight intraperitoneal [14].

I. Superoxide dismutase in erythrocyte lysates [15]

At the end of 21 days of exposure to chronic restraint, blood samples were collected from the rabbits in all groups for the measurement of superoxide dismutase levels in erythrocyte lysates, using commercially available colorimetric assay kits, and an indirect xanthine-xanthine oxidase method, as described by with the results expressed in IU/mL.

II. Measurement of the level of TBARS in nucleus accumbens of tested mice as a marker of lipid peroxidation [16,17]

Nucleus accumbens of each mouse in each group was excised out of the brain and 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 micro liters 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.

III. Effect of lamotrigine on the activities of nucleus accumbens catalase and glutathione peroxidase of tested mice

a) Catalase enzyme activity [18]

Catalase activity in the nucleus accumbens homogenates was assayed colorimetrically using dichtromate-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 [19]

Glutathione peroxidase activity in the nucleus accumbens homogenates was measured. Activity was expressed based on inhibition of glutathione.

c) Protein determination [20]

The total protein content of nucleus accumbens 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.

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.

Data analysis

The results were presented as medians with 25 and 75 percentiles for seizure score and mean ± standard deviation for seizure onset. Data were analyzed using one-way analysis of variance with Tukey test at a 95% confidence level with significant differences between groups at p<0.05.

Results

A) Effect of lamotrigine on PTX-induced convulsions

Single dose lamotrigine treatment significantly (p<0.05) delayed the onset and reduced the severity of PTX-induced convulsions compared with the control groups (1 and 2) (Table 1).
B) Anti-oxidant tests after exposure of the three tested groups of mice to chronic restraint model:

I. Effect of lamotrigine on superoxide dismutase (SOD) enzyme

Single daily dose of lamotrigine for 21 days, during exposure to chronic restraint, significantly (**p<0.05) increased superoxide dismutase enzyme levels compared to both control groups without and with injection of vehicles (88.15 ± 5.6 IU/mL [Group 3] versus 38.98 ± 5.8 and 42.60 ± 2.7 IU/mL [Group 1 and 2]) (Figure 1).

Notes: Results are expressed as mean ± SD (n=12 mice/group). Lamotrigine-treated group (3) significantly (*p<0.05) increased SOD enzyme levels in comparison to the control groups (1 and 2).

Table 1: Effect of treatment with lamotrigine on picrotoxin (PTX)-induced convulsions.

<table>
<thead>
<tr>
<th>Group</th>
<th>Onset of convulsions in seconds</th>
<th>Severity (Score range (1-7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control without vehicles group (1)</td>
<td>430.2 ± 26.67</td>
<td>5 5 1*</td>
</tr>
<tr>
<td>Control injected with vehicles (group 2)</td>
<td>428.9 ± 17.16</td>
<td>5 5 1*</td>
</tr>
<tr>
<td>Group-treated with lamotrigine dissolved in vehicles (group 3)</td>
<td>1376 ± 8484</td>
<td>5 5 2*</td>
</tr>
</tbody>
</table>

Notes: Modulation of onset and severity of PTX-induced convulsions in mice treated with lamotrigine (groups 3). Treatment with lamotrigine significantly (p<0.05) delayed onset of convulsions and reduced their severity score compared with the control groups (group 1 and 2).

II. Effect of lamotrigine on the level of TBARS in nucleus accumbens of tested mice as a marker of lipid peroxidation

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

Figure 1: Effect of lamotrigine on superoxide dismutase (SOD) enzyme content in RBCs lysates of tested albino mice. Exposure to chronic restraint for 21 days of tested mice (Group 1 & 2) significantly (*p<0.05) reduced superoxide dismutase enzyme content in erythrocyte lysates. However, group (3)-treated with lamotrigine showed significant (**p<0.05) increase in its content.

Figure 2: Effect of lamotrigine on the level of TBARS in nucleus accumbens of tested mice as a marker of lipid peroxidation.

III. Effect of lamotrigine on the activities of nucleus accumbens catalase and glutathione peroxidase

Figure 3 shows changes in the activity of nucleus accumbens catalase in different tested groups of mice.

Figure 4 shows changes in the activity of glutathione peroxidase enzyme in the tested nucleus accumbens of different tested groups of mice.

Table 2: Changes in the activity of catalase in nucleus accumbens of tested groups of mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD of catalase in N.A. in Unit/mg tissue protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control without vehicles</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>Control with vehicles</td>
<td>50 ± 2</td>
</tr>
<tr>
<td>Lamotrigine-treated</td>
<td>150 ± 10</td>
</tr>
</tbody>
</table>

Notes: Significant decrease in the activities of these antioxidant enzymes in groups (1 and 2) exposed to chronic restraint model.

While the lamotrigine-treated Group (3) showed that the activities of these enzymes as antioxidants were significantly (**p<0.05) increased compared to control groups (1 and 2).

Changes in the activity of nucleus accumbens catalase in different tested groups of mice.
were decreased by the administrations. The increases of antioxidant and plasma of depression group were elevated although their levels induced depression group. The LP levels in the cortex of the brain increased by the three drugs administrations to the animals of CMS brain although their levels and beta-carotene concentrations were Px) activity, reduced glutathione and vitamin C of cortex of the
resulted in significant decrease in the glutathione peroxidase (GSH-

Discussion

The oxidative stress and modulation of antioxidant enzyme activity may contribute to the central deleterious consequences of chronic stress. One of the neurochemical complications associated with epilepsy is increased lipid peroxide levels, especially TBARS, in the brain [21,22]. Enhanced lipid peroxidation can induce seizure activity by direct activation of glutamine synthase, thereby permitting an abnormal buildup of the excitatory neurotransmitter glutamic acid [23].

The results of the present study revealed that lamotrigine, as an anti-epileptic drug acts by blocking voltage-dependent sodium channels and inhibiting the release of excitatory amino acids. This may stabilize neurons in epileptic foci, reduced the severity of convulsions and seizure score of picrotoxin-exposed mice. As regards its possible anti-oxidant activity in nucleus accumbens of brains of tested mice exposed to chronic restraint model, the results pointed to a decrease in TBARS content with an increase in the activities of both catalase and glutathione reductase enzymes of this area of pleasure. Lamotrigine also increased the level of superoxide dismutase (SOD) enzyme in erythrocyte lysates of tested mice of the present study.

An experimental study investigated the effects of lamotrigine, aripiprazole and escitalopram administration and experimental depression on lipid peroxidation (LP) and antioxidant levels in cortex of the brain in rats. Depression, as well as the oxidative stress, resulted in significant decrease in the glutathione peroxidase (GSH-Ps) activity, reduced glutathione and vitamin C of cortex of the brain although their levels and beta-carotene concentrations were increased by the three drugs administrations to the animals of CMS induced depression group. The LP levels in the cortex of the brain and plasma of depression group were elevated although their levels were decreased by the administrations. The increases of antioxidant values in lamotrigine group were higher according to aripiprazole and escitalopram supplemented groups. Vitamin A level did not change in the five groups. 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 ultra structure 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 pentylentetrazole (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 pentylentetrazole-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 pentylentetrazole-kindled group as compared to the pentylentetrazole-kindled carbamazepine-treated group [26].

In conclusion, the management of epilepsy by lamotrigine could be associated with possible beneficial anti-oxidant actions.

It reduces seizure frequency and intensity in mice exposed to a chemoconvulsive model. Additionally, its anti-convulsant effect could be related to a decrease in levels of the oxidative stress marker TBARS with an increase in catalase and glutathione peroxidase enzymes of nucleus accumbens of brains of tested mice exposed to chronic restraint model.

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Disclosure

The author reports no conflicts of interest in this work.

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