Research Article

Lamotrigine and Memory Impairment Associated with Generalized Seizures: A Study on Maximal Electroshock Seizure Model of Mice

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ABSTRACT

Memory impairment (MI) a category of cognitive disturbance is a common annoyance in epileptic patients. The disease remedy is an anti-epileptic drug (AED) treatment which has been at certain cases found to complicate this issue due its CNS side effects. Here we aim to assess the degree of memory impairment associated with generalized seizures and the effect of lamotrigine (LAM) at normal and reduced dose on this unpleasant effect. Standard phenytoin (PHT) was used for comparing the effect of LAM on MI associated with generalized seizures. Albino mice (n=36) of either sex were treated for a period of 29 days with the drug alone and further combined with levetiracetam (LEV) for 35 days. Assessment of anti-epileptic activity by maximal electroshock on every 7th day and memory impairment activity by Morris water maze (MWM) task on every 8th day for a 64 days treatment schedule was performed. Abolishment of extensor phase, escape latency time (ELT) and time spent in target quadrant (TSTQ) were the parameters observed. Mechanistic studies involved the determination of acetylcholiesterase (AChE) and glutamate levels in brain homogenates. LAM significantly exhibited a lesser degree of memory impairment which was observed by MWM test and decreased AChE and glutamate levels when compared to phenytoin treated group. Combination of LEV was an approach implemented to correct the aggravating effect of MI by the AED, decreased the same significantly and additionally exhibited synergism in anti-epileptic activity. These outcomes could hence aid in the incorporation of LAM and LEV as poly-therapeutic agents and as an alternative to phenytoin to reduce the patient non-compliance to epilepsy treatment. However the need for in-depth research is crucial for the conclusion to be effective for incorporation into the worldly scenario.

Keywords: Generalized epilepsy, memory impairment, acetylcholinesterase, glutamate.

INTRODUCTION

Although various elements such as seizure frequency, seizure type, duration of the disorder and anti-epileptic drug medication may be influential on memory impairment (MI), its inter-relationship is in question till date.[1] Reports of a direct tactic to limit MI are quite rare which makes it an interesting problem to be tackled in epilepsy research. Among the accessible approaches of pharmacology, the utilization of a memory enhancer which also possesses anti-epileptic property was taken into consideration for the present study.

Levetiracetam (LEV) is one such drug exhibiting both memory enhancing and antiepileptic activity.[2,3] Selection of LEV was based on the purpose of its nullity in drug interactions with other AED's.[4]

A broad spectrum AED chosen for this study was lamotrigine (LAM) exhibiting pharmacological actions similar to that of phenytoin (PHT). LAM has been prescribed in cases of paediatric bipolar disorder and attention deficit hyperactivity disorder with studies verifying the substance to improve disease associated neurocognitive profiles.[5,6] PHT an AED which has long been reported to cause impairment in memory function was considered as the standard.[7]

This research focuses on the MI associated with generalized epilepsy as well as the effect of treatment regimen i.e. LAM on this undesirable indication when administered at normal and reduced doses.

Additional to this, the effect of LEV on MI upon combination was assessed.

MATERIALS AND METHODS

Albino mice (n=36) of either sex, 3-6 weeks old ranging in weight of 30-35g were procured from the Central Animal House Facility of JSS Medical College, Mysore, CPCSEA 261/PO/ReBi/2000/CPCSEA, Date: 16/10/2015–15/10/2018.

They were housed in polypropylene cages with free access to food and water, at an ambient temperature 26°C, humidity 50-60%, 12:12 light/dark cycle.

All efforts were made to minimize animal suffering, where experimental protocols were performed in accordance with the approval of Institutional Animal Ethics Committee (IAEC) of JSS College of Pharmacy, Mysuru; bearing the proposal number 145/2014.

PHT, LAM and LEV were procured from Shivari Pharmaceuticals, Mysuru – 570021, Karnataka, India. Maximal Electric Shock (MES) Convulsiometer (INCO electro-Convulsiometer) was used to produce tonic and clonic convulsions by providing electric shocks (45 mA for 0.2 s) via ear electrodes.

Lab made Morris Water Maze (MWM) was employed in

determining MI while Sony Handy cam was utilized to record Escape Latency Time (ELT) and Time Spend in Target Quadrant (TSTQ). Acetylthiocholine iodide, Cage hydrate powder of glutamate and Whatman No. 1 Chromatography paper from Sigma Aldrich, butanol, acetic acid, ninhydrin and cupric sulphate of analytical grade were used, provided by JSS College of Pharmacy, Mysore, Karnataka, India.

Table 1: Treatment Schedule

Group	n	Treatment	Evaluation
Normal	06	0.5% sodium CMC (vehicle) was administered orally for 64 days.	Anticonvulsant activity by MES method on every 7th day and memory impairment activity on every 8th day were noted for 64 days.
Control	06	0.5% Sodium CMC was administered orally every day and convulsions was induced by MES every 7th day for a period of 64 days.	---do---
PHTN + LEV	06	PHT (Normal dose) alone was administered orally for 29 days followed by PHT + LEV for remaining 35 days and convulsions was induced by MES every 7th day for a period of 64 days.	---do---
PHTR + LEV	06	PHT (Reduced dose) alone was administered orally for 29 days followed by PHT + LEV for remaining 35 days and convulsions was induced by MES every 7th day for a period of 64 days.	---do---
LAMN + LEV	06	LAM (Normal dose) alone was administered orally for 29 days followed by LAM+LEV for remaining 35 days and convulsions was induced by MES every 7th day for a period of 64 days.	
LAMR + LEV	06	LAM (Reduced dose) alone was administered orally for 29 days followed by LAM + LEV for remaining 35 days and convulsions was induced by MES every 7th day for a period of 64 days.	---do---

PHTN-Phenytoin Normal dose-24mg/kg, **PHTR-**Phenytoin reduced dose-12mg/kg; **LAMN-**Lamotrigine Normal dose 16mg/kg, **LAMR-**Lamotrigine reduced dose-8mg/kg; **LEV-**Levetiracetam-25mg/kg

Estimation of Brain Acetyl Cholinesterase Activity

Mice, after 64 days were euthanized, decapitated and brains were removed quickly and placed in ice-cold saline. Frontal cortex was quickly dissected out on a Petri dish chilled on crushed ice.

The tissues are weighed and homogenized in 0.1M Phosphate buffer (pH 8).

0.4ml aliquot of the homogenate is added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB.

The contents of the cuvette are mixed thoroughly by bubbling air and absorbance is measured at 412 nm in a spectrophotometer. When absorbance reaches a stable value, it is recorded as the basal reading.

20µl of substrate i.e., acetylthiocholine is added and change in absorbance was recorded. Change in the absorbance per minute is thus determined.[11,12]

The enzyme activity = ΔA*Vt/€bVs

Where ΔA - Change in absorbance, Vt - Total volume (3.1), € - 13610*104, b - Path length (1Cm), Vs - Sample volume (0.4ml)

Estimation of Brain Glutamate Levels

Preparation of Reagents

1. Solvent: Butanol: acetic acid: water (12:3:5):, To 60 ml of butanol, 15 ml of acetic acid and 25 ml distilled water were added.

2. 0.25% Ninhydrin: 200 mg of Ninhydrin was dissolved in 99 ml of acetone. To this solution

1ml of pyridine was added.

3. 0.005% CuSO4 solution: 5 mg of cupric sulphate was dissolved in 10 ml 75% alcohol.

Standards

2µM glutamate: 2.942 mg of glutamate was dissolved in 10 ml of distilled water. After the dissection of different brain regions, each region was homogenized in 80% double distilled ethanol (for every 100mg of the brain tissue, 2ml of 80% alcohol is used).

Homogenates were transferred to polypropylene tubes and centrifuged at 1200rpm for 10 min. 1ml of the supernatant was then transferred into small test tubes and evaporated to dryness at 70˚C in an oven.

The residue was reconstituted in 100 ml distilled water and 10 ml was used for spotting on Whatman No.1 Chromatography paper. Standard solutions of glutamate

at a concentration of 2 mM were also spotted using an Eppendorf micropipette; the spots were dried with a hair drier.

The chromatograms were then stitched at the sides and placed in a chromatography chamber containing butanol: acetic acid: water (65: 15: 25, V/V) as solvent.

When the solvent front reached the top of the papers, the papers were removed and dried.

A second run was performed similarly, after which the papers were dried, sprayed with ninhydrin (0.25% in acetone with 1% pyridine) and placed in an oven at 100˚C for 4 min.

The portions which carry glutamate corresponding with the standard were cut and eluted with 0.005% CuSO4 in 75% ethanol.

Their absorbance was read against a blank in a LKB- 4050 spectrophotometer at 515nm and the levels were expressed as mmoles/gram wet weight tissue.[13]

Calculations:

Glutamate level= Unknown OD*Standard (3 mg)*100/ Standard OD*Volume spotted (10µL)* X;

Where, A = Aminoacid content in umoles/gram wet weight tissue, 1000 = Conversion factor for gram wet weight tissue, X = weight of the tissue in gram

Data and Statistical Analysis

The values expressed in this manuscript are Mean ± SEM, n=6, p≤0.05 analysed by Two-way and One-way ANOVA followed by Tukey's Post Hoc test

RESULTS AND DISCUSSION

Memory Impairment Activity

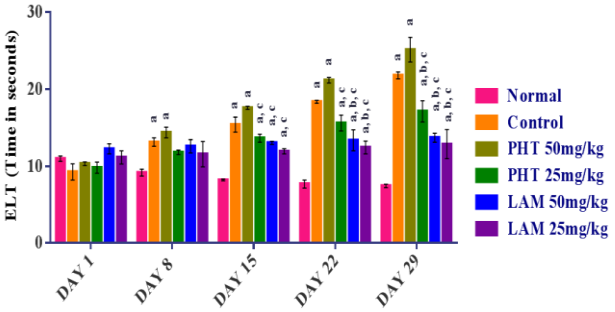
Figure 1 indicates the increase in ELT of control group (21.78±0.45) than healthy or normal group (8.28±0.22; p≤0.05).

A similar increase upon treatment with PHTN i.e. 24mg/kg, was observed in the ELT values (25.12±1.60; p≤0.05). Dose reduction of the same drug i.e. PHTR 12mg/kg reduced the ELT to 17.12±1.37.

The treatment group LAMN 16mg/kg increased the ELT values when compared to normal, but decreased the same than control and PHTN groups (13.69±0.57; p≤0.05).

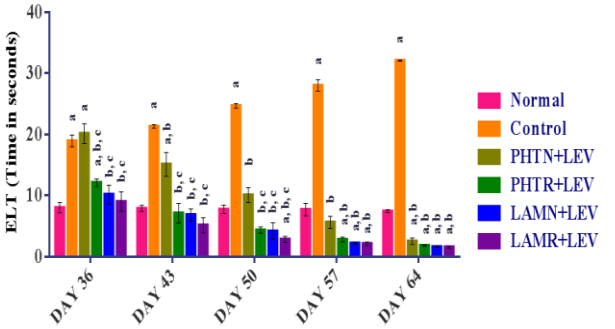
A similar outcome was found for LAMR 8mg/kg on comparison with normal, control and PHTN groups (12.85±1.88; p≤0.05).

For a further study period of 35 days (Figure 2) ELT values considering the control group (32.05±0.10; p≤0.05) an increase on comparison with normal (7.43±0.24) was observed. LEV administration via combination therapy in PHTN, PHTR, LAMN and LAMR has brought about in reversal of ELT when compared to both normal and control (2.51±0.53, 1.80±0.11, 1.58±0.10 and 1.55±0.16; p≤0.05 respectively).



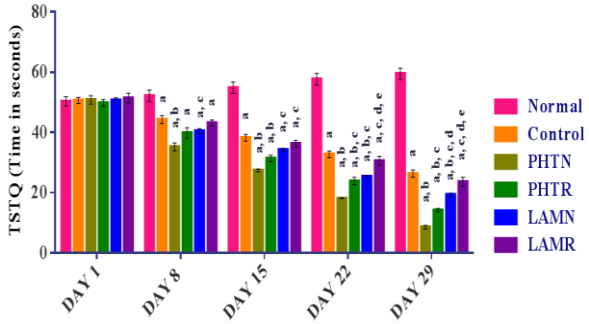
Values are expressed as Mean ± SEM, n=6, p≤0.05 analysed by one-way ANOVA followed by Tukey's Post Hoc test. a- Significant when compared to normal animals, b- Significant when compared to control animals, c- Significant when compared to PHTN animals.

Figure 1: Memory impairment activity of LAM in MES induced convulsive mice (Escape Latency Time in Seconds)



Values are expressed as Mean ± SEM, n=6, p≤0.05 analysed by one-way ANOVA followed by Tukey's Post Hoc test. a-Significant when compared to normal animals, b-Significant when compared to control animals, c-Significant when compared to PHTN+LEV animals.

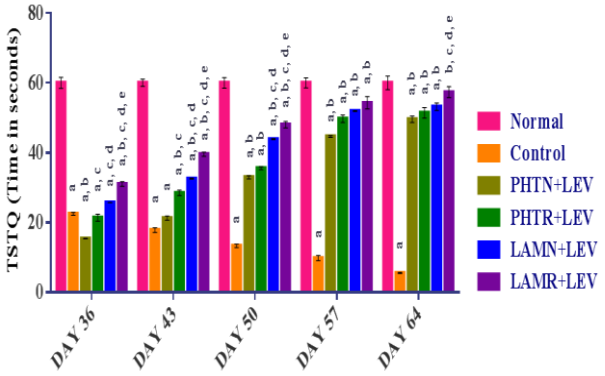
Figure 2: Effect LEV on MI induced by LAM in MES induced convulsive mice (Escape Latency Time in seconds)



Values are expressed as Mean ± SEM, n=6, p≤0.05 analysed by Two-way ANOVA followed by Tukey's Post Hoc test. a- Significant when compared to normal animals, b- Significant when compared to control animals, c- Significant when compared to PHTN animals, d-Significant when compared to PHTR animals, e-Significant when compared to LAMN animals

Figure 3: Memory impairment activity of LAM in MES induced convulsive mice (Time Spent in Target Quadrant in seconds)

TSTQ or Time spent in target quadrant (Figure 3) was a parameter evaluated following ELT. The control values were decreased (26.32±1.24; p≤0.05) when compared to that of normal (59.55±1.88). PHTN further decreased TSTQ than normal and control after 29 days of treatment (8.59±0.60; p≤0.05). PHTR (14.20±0.58; p≤0.05) additionally increased TSTQ values when compared with PHTN. In cases of LAMN and LAMR (19.28±0.47, 23.64±1.47; p≤0.05) the TSTQ findings were less when compared to that of PHT.



Values are expressed as Mean ± SEM, n=6, p≤0.05 analysed by Two-way ANOVA followed by Tukey's Post Hoc test. a- Significant when compared to normal animals, b- Significant when compared to control animals, c- Significant when compared to PHTN+LEV animals, d- Significant when compared to PHTR+LEV animals, e- Significant when compared to LAMN+LEV animals

Figure 4: Effect LEV on MI induced by LAM in MES induced convulsive mice (Time Spent in Test Quadrant in seconds)

The next 35 days (Figure 4) involving the co-administration of LEV with the standard and treatment groups reversed the decrease in TSTQ for the first part of the study i.e. 29 days. An increase was found in PHTN, PHTR and LAMN (49.57±0.94, 51.40±1.48, 53.19±1.07; p≤0.05 respectively) when compared to normal and control. An exquisite observation in LAMR (57.33±1.57; p≤0.05) was its increased TSTQ values when compared to control, PHTN, PHTR and LAMN.

MI in mice was evaluated using MWM which has been widely used in assessing learning and memory.[14]

A simple interpretation of the parameters assessed in this model is as follows: Decreased ELT and increased TSTQ are behavioural indicators of good memory. Increased ELT and decreased TSTQ in the control group were of significance when compared to the normal group thus validating the fact that MI is associated with epilepsy.

A further increase in ELT and TSTQ decrease was observed in PHTN and PHTR which was found to be significant when compared to the control group.

This supports the fact that MI is aggravated by AED's. Reduction in the degree of MI caused was achieved upon dose reduction. However the addition of LEV to the treatment had a greater significance than reduced dose of the AED monotherapy. The degree of MI observed was in the following order: PHTN>PHTR>LAMN>LAMR.

Anti-epileptic Activity

Table 2: Anti-epileptic activity of LAM in presence and absence of LEV on mice by MES induced convulsions (Percentage protection)

Phases	Day	PHTN	PHTR	LAMN	LAMR
		% Protection			
TF*	DAY 1	85.13	71.47	88.74	80.06
TE*		100.00	100.00	100.00	100.00
CC*		87.96	80.58	90.75	88.37
S*		82.98	78.99	83.96	82.14
TF*	DAY 28	86.32	84.31	89.81	84.29
TE*		100.00	100.00	100.00	100.00
CC*		90.62	89.26	92.12	89.48
S*		85.17	83.45	85.55	83.14
TF#	DAY 63	96.88	96.77	95.37	89.88
TE#		100.00	100.00	100.00	100.00
CC#		92.67	91.58	93.59	90.41
S#		86.31	84.67	86.26	83.79

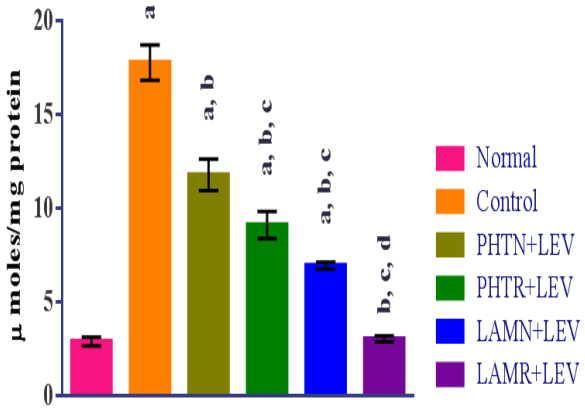
TF-Tonic Flexion, TE-Tonic extension, CC-Clonic convulsions, S-Stupor.

*Indicates administration of AED's alone; #indicates administration of AED's in combination with LEV

Administration of AED's alone for a period of 28 days had profound effects on convulsive phases (Table 2). It was found that LAM had a major influence with respect to the AED activity when compared to PHT. The major emphasis to be explained is that combination therapy involving LEV had a vital effect on the potency of AED's on dose reduction.

The MES model has endured throughout the past years for its place as one of the ideal methods used in the screening of AED's.[15] This study provided us findings which authenticates the treatment therapy as an achievement where LEV could be co-administered in MI aggravating conditions of epilepsy without intervening with the anti-convulsant activities of the drugs employed for treatment. Table 2 describes all the selected AED's to be potent in their activity where LAM had an upper hand in the anti-epileptic activity. However there was marked reduction in the same activity upon dose reduction in order to achieve a lesser degree of MI. This was significantly corrected when LEV was combined with LEV. Not only achieved an attenuation of MI, the anti-convulsant activity was synergized.

Brain Acetylcholiesterase



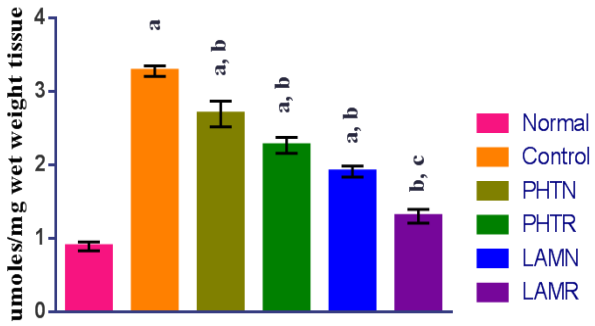
Values are expressed as Mean ± SEM, n=6, p≤0.05 analysed by One-way ANOVA followed by Tukey's Post Hoc test. Significant when compared to normal animals, b- Significant when compared to control animals, c-Significant when compared to PHTN+LEV animals, d- Significant when compared to LAMN+LEV animals

Figure 5: Effect of LAM plus LEV on brain AChE levels in MES induced convulsive mice (After 64 days)

In Figure 5 AChE levels were increased in all the cases of treatment i.e. PHTN, PHTR and LAMN (15.78±0.91, 12.75±0.62 and 11.96±0.79; p≤0.05 respectively) apart from the control group where LAMR (11.05±0.50) exhibited least increase in AChE levels. Figure 5 represents the combination study with LEV on PHTN, PHTR, LAMN and LAMR (11.78±0.84, 9.11±0.72, 6.96±0.18, 3.05±0.15; p≤0.05) were AChE levels were decreased.

The neurotransmitter acetylcholine has always been linked proportionately to memory.[16] A significant decrease was detected when combined with LEV representing the defence mechanism of LEV on MI worsening on an already increased AChE levels by AED's.

Brain Glutamate



Values are expressed as Mean ± SEM, n=6, p≤0.05 analysed by One-way ANOVA followed by Tukey's Post Hoc test. a-Significant when compared to normal animals, b- Significant when compared to control animals, c- Significant when compared to LAMN+LEV animals

Figure 6: Effect of LAM plus LEV on brain Glutamate levels in MES induced convulsive mice (After 64 days)

Increased glutamate (Figure 8) by LEV was found in all cases of the treatment with highest reduction in LAMR (1.30±0.09; p≤0.05) when compared to control (3.28±0.07) and LAMN+LEV (1.91±0.07).

A neurotoxic property of glutamate (excitatory neurotransmitter) above normal levels could be another factor prompting MI.[17] LEV on co-administration reversed the increase in glutamate levels.

CONCLUSION

Depending on the results and an interpretation through discussion, the investigation has come to a conclusion that MI is associated with epilepsy and is worsened when treated with AED's, the cause of which may perhaps exist due to cholinergic and glutaminergic pathway intervention.

Although the degree of MI was reduced on dose reduction of the AED, its potency was affected. In order to tackle this, an approach put forward by combining a memory enhancer additionally possessing anti-convulsant property was successful equally alleviating the condition of MI as well as substituting the lost potency due to dose reduction of AED.

Since LAM was found to have the lesser adverse effect of MI aggravation and a better convulsive protection than PHT, integration of LAM as a first line in the management of epilepsy and as an alternative to PHT to reduce the patient noncompliance to MI can be attained. This tactic could be effective only on an extensive advanced research which is very much essential for the implementation into real-world scenario.

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