Effect of L-Arginine Supplementation on Seizure Severity and Comorbid Cognitive Impairment in Pentylenetetrazole-Induced Epilepsy

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ABSTRACT
Background: Cognitive impairment is a common comorbidity in epilepsy with no definitive treatment. L-arginine has been shown to improve cognition in experimental animals.
Objective: This study investigated the effect of L-arginine on seizure severity and cognition in pentylenetetrazole-induced epilepsy.
Materials and Methods: Forty-two male albino rats were administered pentylenetetrazole (PTZ) intraperitoneally at a dose of 35 mg/kg thrice weekly for 6 weeks. Kindled rats were divided into 5 treatment groups: DW; control administered distilled water at 10 mL/kg, LMT; lamotrigine at 62 mg/kg daily, CBZ; carbamazepine at 93 mg/kg twice daily, L-ARG; L-arginine at 100 mg/kg daily, and L-ARG+CBZ; L-arginine at 100 mg/kg daily plus carbamazepine 93 mg/kg twice daily orally. At 4 weeks on treatment, antiseizure effect was tested using sub-convulsive dose of PTZ. At week 5, cognition was assessed using Y-maze and Morris Water-Maze (MWM) tests. Serum samples were obtained for nitro-oxidative stress parameters assay at week 6.
Results: There was no significant difference in seizure severity in the L-ARG versus control groups. However, L-ARG+CBZ significantly (p<0.001) increased seizure latency and reduced (p<0.05) Racine stage and seizure duration compared to DW. L-ARG and L-ARG+CBZ also significantly increased (p<0.05) percentage correct alternation and reduced (p<0.01) escape latency versus DW. There were no significant differences in serum nitro-oxidative stress parameters between the groups.
Conclusion: L-arginine potentiated the antiepileptic effect of carbamazepine and improved cognition in epileptic rats. L-arginine may be repurposed for managing cognitive dysfunction in epilepsy, but the precise mechanism(s) of action needs to be investigated.

Keywords: Epilepsy, Cognition, Pentylenetetrazole, Memory, L-Arginine.

INTRODUCTION
Epilepsy is a chronic debilitating neurological disease that is characterized by recurrent unprovoked seizures with comorbid neurocognitive and behavioural disorders (1). About 50 million people are affected globally, and majority of those affected are from sub-Saharan African countries like Nigeria (2). Over the past years, several drugs have been developed for the management of epilepsy, each with its unique antiepileptic property and adverse effect profile, but about half of patients continue to experience side effects because of therapy (3–6).

Cognitive impairment (CI) is one of the common adverse effects associated with the use of antiepileptic drugs; it also occur consequent to the pathobiology of epilepsy (7, 8). Cognitive impairment is highly prevalent among people with epilepsy; a study found that 50% of the patients with epilepsy had CI (9, 10). The burden of cognitive impairment is huge; it is a major determinant of quality of life, adherence to therapy and achievement of life goals (10). It has been reported that patients with epilepsy are less likely to be educated compared to their mates, siblings and parents (11). They are more likely to have lower grade point average in school and less likely to be employed compared to the general population (11, 12). Recently a study documented that people with epilepsy are at an increased risk of developing dementia compared to the general population (13).

Despite the huge impact of CI on people living with epilepsy, there is no definitive management of the condition. This is possibly because the mechanism of CI in epilepsy is yet to be fully understood. Available evidence shows that there is shared structural and functional abnormalities in the brain in both epilepsy and cognitive dysfunction, and the specific abnormality linked with cognitive impairment is unknown and this has forestalled targeted therapy (14). Other possible mechanisms for CI in epilepsy include increased oxidative stress, changes in the levels and distribution of neurotransmitters, changes in receptor expression, neuronal reorganization, and
All the experiments were performed during the light phase to allow for acclimatization to the new environment. The animals were brought to the laboratory at least two hours before experiments to maintain ad libitum and kept under controlled environments (18). Carbamazepine is one of the antiepileptic drugs (AEDs) associated with CI and one of the most prescribed antioxidant medications (18).

The role of antioxidants has been well studied in the prevention of post-ictal cognitive impairment and several studies have documented the protective effect of antioxidants in epilepsy associated cognitive impairment (17). L-arginine is an antioxidant and a nitric oxide donor; it has been shown to improve cognition via nitric oxide and non-nitric oxide dependent pathways in non-epilepsy related cognitive impairment (19). It has earlier been reported that L-arginine improves carbamazepine associated cognitive impairment in non-epileptic rats, although it appears that the mechanism may not be related to its antioxidant activity (20). The positive effect of L-arginine on cognitive function and carbamazepine induced CI makes L-arginine an attractive drug for the management of CI in epilepsy, suggesting that L-arginine may be repurposed for the management of cognitive dysfunction in epilepsy. Repurposing L-arginine for cognition in epilepsy may provide a faster and cheaper means of addressing CI in epilepsy without going through the arduous process of developing new therapies.

One of the major concerns of developing drugs for the management of cognitive impairment is the risk of worsening seizure severity and this has been expressed with the use of anticholinesterases which are currently being investigated for possible repurposing for the treatment of cognitive impairment in epilepsy (21–23). The antiseizure effect of L-arginine has been documented although some studies have shown conflicting results (24–27).

Several studies have investigated the use of several interventions in the management of cognitive impairment in epilepsy; however, most of these studies were done in the immediate post-ictal period and these may not be translatable to comorbid inter-ictal cognitive impairment. Therefore, this study investigated the effect of L-arginine as monotherapy and in combination with carbamazepine on seizure severity and comorbid cognitive impairment in PTZ-induced epilepsy in male rats and the relationship with oxidative stress parameters.

MATERIALS AND METHODS

Experimental Animals

A total of 42 healthy male albino rats were obtained from the Animal House of College of Medicine, University of Lagos, Lagos, Nigeria. The animals were housed in plastic cages with access to pelleted rat chow (Top Feed Animal Feeds, Lagos, Nigeria) and water ad libitum and kept under controlled environmental conditions (temperature 22±2°C, humidity: 50–55%, natural light/day cycle). During the period of the experiment, animals were brought to the laboratory at least two hours before experiments to allow for acclimatization to the new environment. All the experiments were performed during the light phase between 9:30am and 12.00noon. The working protocol was approved by the College of Medicine, University of Lagos Animal Care and Use Research Ethics Committee (ACUREC) with number CMUL/ACUREC/05/21/847.

PTLENDETETRAZOL-INDUCED SEIZURES

Pentylenetetrazole (PTZ) powder (Sigma-Aldrich St. Louis, MO, USA) was dissolved in 0.9% saline and administered in a subconvulsant dose of 35 mg/kg body weight intraperitoneally (i.p.) on every alternate day (i.e. Day 1, Day 3, Day 5, etc.) over 6 weeks or until the rats showed adequate kindling i.e. seizure score of 5 on three consecutive injections (28, 29). After each PTZ injection, animals were monitored for 30 min for convulsive behaviour. The resultant seizures were scored as follows: Stage 0 (no response); Stage 1 (hyperactivity, restlessness and vibrissae twitching); Stage 2 (head nodding, head clonus and myoclonic jerks); Stage 3 (unilateral or bilateral limb clonus); Stage 4 (forelimb clonic seizures); and Stage 5 (generalized clonic seizures with falling) (30). Rats that had Stage 5 Racine score on three consecutive administration of subconvulsive doses of PTZ were considered epileptic. Seizures were terminated with intraperitoneal diazepam at a dose of 62 mg/kg (Human equivalent dose (HED) of 10 mg/kg) at 2 min to prevent mortality from prolonged seizures. Thirty rats (85%) were kindled at 6 weeks after a total of 15-18 subcutaneous doses of PTZ.

Animal Groups and Dosing

The rats were divided into five treatment groups consisting of six rats per group after kindling - DW; Distilled water was administered as vehicle at 10 mL/kg daily in the control group, LMT; Lamotrigine (positive control) was administered at the dose of 62 mg/kg daily, CBZ; Carbamazepine (CBZ) at 93 mg/kg twice daily, L-ARG; L-arginine at 100 mg/kg daily, and L-ARG+CBZ; L-arginine at 100 mg/kg daily plus CBZ 93 mg/kg twice daily.

The drugs were administered orally on alternate days (i.e. Day 1, Day 3, Day 5, etc.) for 6 weeks or until the rats showed adequate kindling i.e. seizure score of 5 on three consecutive injections (28, 29). After each PTZ injection, animals were monitored for 30 min for convulsive behaviour. The resultant seizures were scored as follows: Stage 0 (no response); Stage 1 (hyperactivity, restlessness and vibrissae twitching); Stage 2 (head nodding, head clonus and myoclonic jerks); Stage 3 (unilateral or bilateral limb clonus); Stage 4 (forelimb clonic seizures); and Stage 5 (generalized clonic seizures with falling) (30). Rats that had Stage 5 Racine score on three consecutive administration of subconvulsive doses of PTZ were considered epileptic. Seizures were terminated with intraperitoneal diazepam at a dose of 62 mg/kg (Human equivalent dose (HED) of 10 mg/kg) at 2 min to prevent mortality from prolonged seizures. Thirty rats (85%) were kindled at 6 weeks after a total of 15-18 subcutaneous doses of PTZ.

Assessment of Cognition

Cognition was assessed in the epileptic rats at week 5, a week after the antiepileptic effect of drugs were assessed to create time for full resolution of post-ictal cognitive impairment. The groups were evaluated for cognitive function using Y-maze and Morris Water maze (MWM) tests.
Assessment of Percentage Correct Alternation Using Y Maze

Spatial working memory was determined by the percentage correct alternation on the Y-maze apparatus in this study. Each rat was placed at the centre of the Y-maze and allowed to move freely into the different arms for a duration of 4 min to determine locomotor activity and spatial working memory. The animals were pre-exposed 24 hours before the main test; this involves allowing the rats to roam freely in the maze for 2 min. Animals that did not move around were excluded from the test. The apparatus was cleaned with 5% alcohol and allowed to dry between sessions (31).

Locomotor activity was determined by the number of arm entries. Arm entry was defined as the entry of the head and all limbs into an arm (32). An alternation is defined as entry into all three arms consecutively. Percentage correct alternation was determined by dividing the number of maximum spontaneous alternations by the total number of arms entered minus two (31). Percentage correct alternation measures spatial working memory which requires an intact prefrontal cortex (33).

Assessment of Escape Latency Using Morris Water Maze

Morris water test assess the spatial memory by measuring escape latency. The animals were placed in a large pool of a black water tank measuring 1 m in circumference. The tank was roughly divided into 4 equal quadrants and an escape platform was placed 2 cm below the water surface in one of the quadrants at the same position throughout the experiment. The platform was painted black (same shade as the tank) to eliminate visual cues in locating the platform (34, 35).

At the beginning of the trials, the rats were placed into the water tank facing the wall of the water tank at three different locations. The rats were allowed to swim randomly to locate a hidden platform; if the platform was not located in 60 s, the rats were gently guided to the platform and left to stay for 10 s. The time taken (escape latency) to locate the submerged platform was documented for each rat. Measurement of escape latency was carried out after 4 days of consecutive trials which consists of 3 trainings per day at intervals of 15 min (36).

Blood Sample Collection

The rats were sacrificed by cervical dislocation at week 6 of drug administration and blood samples were obtained by cardiac puncture using plain sample bottles. The samples were centrifuged at 4000 rpm for 20 min after which the serum obtained was stored at −20°C for further biochemical analysis of nitric oxide oxidation products which are nitrite NO2− and nitrate NO3−. The levels of reduced glutathione (GSH), malondialdehyde (MDA) and activities of superoxide dismutase (SOD) and catalase (CAT) were also determined in serum.

Determination of Nitrite and Nitrate in Serum of Experimental Rats

The nitrite and nitrate concentrations in the samples were determined by a modified Griess Reagent spectrophotometric method (37). The samples were prepared by adding 1 mL of serum to 9 mL of distilled nitrate free water to mark up to 10 mL. To determine the level of nitrite, 10 mL of the diluted sample was transferred into a separate 50 mL volumetric flask and diluted with 20 mL of deionized water. The resulting solution was then completely shaken on a reciprocating shaker to completely mix, and 2.5 mL of sulfuric acid solution was added and shaken for 5 min, after which 2.5 mL of N-(1-Naphthyl) ethylenediamine dihydrochloride solution was added. This solution was also shaken to achieve homogeneity. The solution was then made to mark with deionized water, and its absorbance was measured at 540 nm. Standard nitrite solutions of 0.1–2.0 mg/L (25 mL each) prepared from freshly dried NaNO2 were also similarly treated. The nitrite concentrations of the samples were obtained from the prepared calibration curve of the standard solutions, after taking the dilution factor into consideration.

A similar aliquot was prepared to determine the serum concentration of nitrate; after adding 20 mL of deionized water, the solution was shaken on the reciprocating shaker for 5 min. 0.1 g of zinc powder and 1 mL of hydrochloric acid solution were then added to the preparation. The mixture was allowed to stand for 10 min (to reduce nitrate to nitrite). Afterwards, 2.5 mL of sulfuric acid solution was added, shaken until homogeneously mixed and left to stand for 5 min. Thereafter, N-(1-Naphthyl) ethylenediamine dihydrochloride solution (2.5 mL) was added and the mixture was then made to mark with deionized water. The absorbance of the solution was measured at 540 nm. Standard solutions of 0.1–2.0 mg/L (25 mL each) prepared from freshly dried NaNO3 were also similarly treated. The concentration of total nitrate (nitrite and converted nitrate) was calculated using the regression equation from the calibration curve obtained and the dilution factor. The concentration of converted nitrate (nitrate that has been converted to nitrite) was obtained by subtracting the concentration of nitrite from the total nitrite obtained for each sample.

The total concentration of nitric oxide oxidation product (nitrite and nitrate) was used as surrogate markers for serum nitric oxide levels (38).

SOD Activities in Serum of Experimental Animals

SOD activity was measured using quercetin as the substrate after suitable dilution method as previously described (39). The assay mixture in a total volume of 1 mL consisted of 0.1 mol/L sodium phosphate buffer (pH 7.8) and 0.08 mmol/L EDTA at a proportion of 1:1. The 0.1 mL of sample (1:1000) after dilution was added to 2.3 mL of distilled water, after which 1 mL of assay mixture with EDTA and sodium phosphate buffer was added. The increase in absorbance due to oxidation of quercetin at 0 and 20 min were measured spectrophotometrically at 406 nm. In the blank, sample was substituted by equal quantities of distilled water. One unit of SOD activity is defined as the quantity of enzyme that inhibited quercetin oxidation by 50% under given experimental conditions.

CAT Activities in Experimental Animals

CAT activity was assayed according to the method of Koroliuk et al. (40). The reaction was started by the addition of 0.1 mL of serum to 1 mL of 4% ammonium molybdate and 2 mL of 0.03% H2O2 solution. One unit of catalase activity is defined as
the amount of enzyme required to clear 1 μmol of H2O2 per minute per gram of tissue. The breakdown of hydrogen peroxide in the reaction mixture was measured spectrophotometrically at 410 nm (40).

**GSH Levels in Experimental Animals**

GSH measurement was determined spectrophotometrically by measuring NADPH oxidation at 340 nm. The reaction mixture contained sample, 0.1 mM NADPH, 0.5 mM EDTA, 0.1 M potassium phosphate (pH 7.5) and 200 mM KCl. After 5 min of pre-incubation at 37°C, the reaction was initiated by addition of 1 mM GSSG (41).

**MDA Levels in Experimental Animals**

Estimates of lipid peroxidation levels were evaluated by the thiobarbituric acid reactive substances (TBARS) procedure, described by Timirbulatov and Selezniev (42). This method involves reaction of thiobarbituric acid (TBA) with the degradation product of lipid peroxidation, MDA, under conditions of high temperature and acidity to generate a coloured adduct that is measured spectrophotometrically. 2 mL of distilled water was added to 0.1 mL of sample, which was followed by 1 mL of TBA reagent and 1 mL of trichloroacetic acid. The mixture was heated in a boiling water bath for 10 min before the addition of butanol. After cooling, the mixture was centrifuged for 10 min. Absorbance in the organic phase was determined at 532 nm and samples were compared to a blank (42).

**Statistical Analysis**

All results are expressed as mean ± standard error of mean (S.E.M.). The data were statistically compared using one way ANOVA followed by Tukey’s post-hoc test for inter-group comparisons. Statistical analysis was carried out using GraphPad Prism software version 6.0 (GraphPad Software, San Diego, CA, USA). P value of less than 0.05 was considered significant for comparison.

**RESULTS**

**Seizure Severity in Experimental Animals**

The effect of L-arginine on seizure severity was determined by measuring seizure latency, Racine stage and seizure duration in the experimental animals. Seizure latency was significantly prolonged in CBZ and L-ARG+CBZ groups compared to the DW group (p<0.01 and 0.001, respectively). Seizure latency was also higher in CBZ and L-ARG+CBZ groups compared to the LMT group (p<0.01). There was no significant difference in values for L-ARG compared to DW and LMT. The seizure latency in L-ARG+CBZ group was significantly higher compared to the CBZ (p<0.05) and L-ARG (p<0.05) groups (Figure 1).

There was no significant difference (p>0.05) in the Racine stage between DW, LMT and L-ARG groups. Racine stage was however significantly lower in the CBZ and L-ARG+CBZ groups versus DW group (p<0.05 and 0.001 respectively). The Racine stage was also lower in the L-ARG+CBZ group versus LMT group (Figure 2).

The seizure duration was not significantly different (p>0.05) in the DW, LMT and L-ARG treated groups. Seizure duration was however significantly shorter in the CBZ (p<0.05) and L-ARG+CBZ groups (p<0.01) versus DW group (Figure 3).

**Cognition in Experimental Animals**

In assessing cognition using Y-maze, there was no significant difference (p>0.05) in locomotor activity between all the treatment groups versus control (Figure 4). Measurement of percentage correct alternation showed that the L-ARG and L-ARG+CBZ groups had significantly higher percentage correct alternation compared to DW group (p<0.05 and 0.01 respectively). There was no significant difference in the other groups (Figure 5).

In the Morris water-maze experiment (Figure 6), the escape latency was significantly reduced in the LMT (p<0.05), L-ARG (p<0.001) and L-ARG+CBZ (p<0.01) groups compared to DW group. There was no significant difference between the CBZ and DW groups. L-ARG and L-ARG+CBZ groups had significantly lower escape latency compared to CBZ group (p<0.01, and 0.001 respectively).

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**Fig. 1: Effect of L-arginine on Seizure Latency in PTZ-induced Epilepsy.**

Values are presented as mean ± SEM, n = 6 for each group. *p<0.001 vs. DW control, †p<0.001 vs. LMT, ‡p<0.05 vs. CBZ, and §p<0.05 vs. L-ARG+CBZ (one way ANOVA followed by Tukey’s post hoc test).

**Fig. 2: Effect of L-arginine on Racine stage in PTZ-induced Epilepsy.**

Values are presented as mean ± SEM, n = 6 for each group. *p<0.05, †p<0.001 vs. DW control, ‡p<0.01 vs. LMT (one way ANOVA followed by Tukey’s post hoc test).
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Serum Levels of Nitrite, Nitrate and Nitric Oxide in Experimental Animals
There was no significant difference ($p > 0.05$) in the levels of nitric oxide oxidation products (nitrite and nitrate) and calculated nitric oxide (nitrite + nitrate) in all the treatment groups versus DW control.

Serum Oxidative Stress Parameters in Experimental Animals
There was no significant difference ($p > 0.05$) in the serum levels of CAT, GSH, MDA and SOD in all the treatment groups versus control.

Table 1: Effect of Treatments on Serum Levels of Nitrite and Nitrate in Experimental Animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nitrite $\text{NO}_2^-$ (mg/l)</th>
<th>Nitrate $\text{NO}_3^-$ (mg/l)</th>
<th>Nitric Oxide $\text{[NO}_2^-+\text{NO}_3^-]$ (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>2.64±0.8</td>
<td>4.99±0.41</td>
<td>7.36±1.27</td>
</tr>
<tr>
<td>LMT</td>
<td>2.16±0.19</td>
<td>3.96±1.47</td>
<td>6.09±0.62</td>
</tr>
<tr>
<td>CBZ</td>
<td>1.81±0.42</td>
<td>4.99±1.41</td>
<td>5.10±2.25</td>
</tr>
<tr>
<td>L-ARG</td>
<td>1.7±0.27</td>
<td>4.25±2.3</td>
<td>5.35±2.01</td>
</tr>
<tr>
<td>L-ARG+CBZ</td>
<td>2.74±0.49</td>
<td>5.62±0.49</td>
<td>6.67±0.58</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM, $n = 6$ for each group. $p > 0.05$ vs. DW control (one way ANOVA followed by Tukey’s post hoc test).

Table 2: Effect of Treatments on Serum Oxidative Stress Markers in Experimental Animals

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>CAT (U/ml)</th>
<th>GSH (nmol/ml)</th>
<th>MDA (nmol/ml)</th>
<th>SOD (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>26.8±0.92</td>
<td>10.08±0.73</td>
<td>2.05±0.10</td>
<td>4.15±0.28</td>
</tr>
<tr>
<td>LMT</td>
<td>32.13±5.95</td>
<td>9.76±0.58</td>
<td>1.85±0.17</td>
<td>4.18±0.22</td>
</tr>
<tr>
<td>CBZ</td>
<td>28.33±0.74</td>
<td>8.60±0.31</td>
<td>2.35±0.17</td>
<td>3.43±0.06</td>
</tr>
<tr>
<td>L-ARG</td>
<td>32.5±1.25</td>
<td>9.52±0.37</td>
<td>2.33±0.21</td>
<td>3.9±0.11</td>
</tr>
<tr>
<td>L-ARG+CBZ</td>
<td>29.77±1.03</td>
<td>8.96±0.44</td>
<td>2.28±0.34</td>
<td>3.88±0.24</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM, $n = 6$ for each group. $p > 0.05$ vs. DW control (one way ANOVA followed by Tukey’s post hoc test).
DISCUSSION

The study demonstrated that subacute administration of L-arginine had no anticonvulsant effect when used as monotherapy but had a synergistic effect when used in combination with carbamazepine (43). Only a few studies have investigated the prolonged use of L-arginine on seizure control in epileptic rats. One of the few studies demonstrated that L-arginine had no effect on seizure severity in premenopausal female rats that were treated for 4 weeks (43), which is in consonance with the findings in this study. In humans, a study which used L-arginine as add on therapy in patients with poor seizure control demonstrated a significant reduction in seizure frequency (44). This report suggests a synergistic role for L-arginine, which is similar to the findings in this study. Most studies have investigated the role of L-arginine in acute seizure models and results have shown both anticonvulsant and proconvulsant effects (24–27).

It has been shown that L-arginine's proconvulsant activity in PTZ-induced acute seizures may be due to excessively high levels of NO, since elevated levels of NO play a role in the proconvulsant activity of PTZ (45, 46). However, this study did not find an increase in basal levels of nitrate or nitrite following the subacute administration of L-arginine; this may explain the neutral effect of L-arginine on seizure severity. The unchanged levels of nitrate or nitrite with L-arginine administration in this study may be due to the low dose administered or tolerance which has been documented with prolonged use of L-arginine (47–49). L-arginine dose of about 60 mg/kg has been reported as non-effective dose (47, 48). Finally, tolerance to the effect of L-arginine with prolonged use has been documented and may explain the relatively low levels (49). This however needs to be proven in further studies.

The synergistic effect observed between L-arginine and carbamazepine in this study has been documented with some drugs and herbal agents (50–52). Some studies on PTZ-induced seizures have demonstrated that L-arginine supplementation improved the anticonvulsant effect of essential oil derived from the leaves of Zhumeria majdae, pioglitazone and levosimendan (50–52). Other studies however, documented a dose-dependent proconvulsant effect of L-arginine combination (47, 48, 53). It appears that the effect of L-arginine is determined by the properties of the added drug, therefore suggesting a neuromodulatory role.

Neuromodulation involves the alteration of cellular or synaptic properties of a neuron using various methods to cause a change in the excitability or the strength of a synapse. Neuromodulation is achieved by using chemical, electrical, magnetic and thermal methods (54). The modulatory effects of both L-arginine and NO have been documented and may play a role in potentiating the action of carbamazepine in this study (43, 55). Potentiation is a kind of synergistic drug reaction in which a drug which lacks a specific pharmacological action enhances the activity of another drug with that same pharmacological property (56). Further studies need to be carried out to fully understand the neuromodulatory effect of L-arginine.

This study demonstrated that L-arginine improved comorbid cognitive function in epileptic rats in monotherapy and in combination therapy. Carbamazepine is known to be associated with cognitive impairment (18); in this study, cognitive dysfunction was however not worse in the control group compared to carbamazepine. The reason for this is not apparent; it could be argued that the methods used for cognitive impairment in this study may not be sensitive to the added effect of carbamazepine. Further studies need to be done to confirm this. Nevertheless, the supplementation of carbamazepine with L-arginine significantly improved cognition. It has been reported that L-arginine improved cognition in carbamazepine associated CI in non-epileptic rats (20). It is well known that poor seizure control and more severe seizure are associated with worse cognitive profile in humans (57). The effect of L-arginine on cognition observed in this study is independent of its effect on seizure severity since it had a similar profile as monotherapy and when used to supplement carbamazepine use.

Increased nitro-oxidative stress has been documented as a contributor to cognitive impairment, as well as in the process of epileptogenesis, and several studies have demonstrated the beneficial effect of drugs and herbal therapies in improving post-ictal cognitive impairment in PTZ-kindled epileptic rats (58–60). The study investigated the effect of L-arginine on oxidative stress parameters to determine if the cognition enhancing property was related to its antioxidant properties. This study showed no significant change in serum nitro-oxidative parameters in the L-arginine group compared to the other treatment groups. Thus, suggesting that increased oxidative stress may not be responsible for the comorbid cognitive impairment in the epilepsy model. The study measured nitro-oxidative parameters more than one week from the last epileptic seizure to eliminate the effect of changes associated with acute seizures. Most studies have measured oxidative parameters in the immediate post-ictal period or less than 24 hours post seizures.

Data on oxidative parameters and comorbid cognitive impairment in epilepsy is sparse in animal models. Nevertheless, this study suggests that oxidative stress may not be a significant player in the mechanism of action of L-arginine in improving cognition. L-arginine has been shown to possess pleiotropic properties; it acts through multiple mechanisms to cause pharmacological changes in different organ-systems of the body (61). Its action has been linked mainly to its NO generating property has been used in a significant number of studies to cause oxidative stress in cell cultures (61). Changes in protein and receptor expression leading to improved cognitive function has also been reported with the use of L-arginine in non-epilepsy models of cognitive impairment, and this may or may not be associated with NO generation (19). These alternate pathways may explain the pharmacological actions in this PTZ model of comorbid cognitive impairment.

Another possible mechanism of action of L-arginine may be through other intermediate products of its metabolism. L-arginine has many intermediate molecules such as ornithine, proline, polyamines, creatine and agmatine, and some of these
molecules have been shown to have distinct pharmacological properties (62, 63). Investigating the effect of these intermediate molecules on seizures may shed better understanding on the role of L-arginine in epilepsy.

This study demonstrated that L-arginine potentiated the antiepileptic effect of carbamazepine and improved interictal cognitive impairment in PTZ-induced epilepsy. This study did not find any relationship between L-arginine administration and nitro-oxidative stress parameters, suggesting that the effect on seizure severity and cognition may not be due to nitro-oxidative stress or low levels of NO. Several studies have investigated the role of L-arginine in seizures, but the results cannot be extrapolated to this study because of major differences in models, dose and methodology of research. This study was carried out in an established epilepsy model with animals treated for six weeks in contrast to acute seizure models. Acute seizures are symptomatic of a disruption leading to increased excitatory and reduced inhibitory neurotransmission, whereas seizures in epilepsy have a combination of changes in neurotransmission on a background of altered neuronal network and synapses which leads to a predisposition to continue to generate epileptic seizures.

CONCLUSION

The study demonstrated the synergistic effect of subacute administration of L-arginine with carbamazepine on seizure control and cognition-enhancing properties in the interictal period. The effect appears not to be mediated by nitric oxide dependent mechanism since there was no difference in the levels of nitrite and nitrate across the treatment groups. This suggests a modulatory role of L-arginine for cognition and its antiepileptic effect in combination therapy. Further studies are needed to determine the actual mechanism of its modulatory effect.

Conflict of Interest

The authors have no conflict of interests to declare.

REFERENCES

L-arginine Supplementation and Cognition in Epilepsy


