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Add-on Effect of Levetiracetam on Cognitive Activity of Carbamazepine and Topiramate Treated Healthy Rats

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ABSTRACT

Background: Many antiseizure drugs are used for non-epileptic indications like bipolar disorder, anxiety, neuropathic pain, prophylaxis of migraine, etc. Cognitive problems are known with many of these agents in epileptic situations but not to that extent in other situations. The antiepileptic Levetiracetam has been shown to improve a range of cognitive abilities.

Objective: To study the effect of levetiracetam, carbamazepine, topiramate and co-administration of levetiracetam with carbamazepine and topiramate on cognition in healthy rats.

Materials and Methods: Wistar albino rats of either sex were randomly assigned to 6 groups (n=6). Treatment groups: I - Normal saline; II, III & IV- Levetiracetam (180mg/kg), Carbamazepine (50mg/kg) and Topiramate (20mg/kg) respectively; V & VI- Levetiracetam + Carbamazepine and VI- Levetiracetam + Topiramate respectively orally for 21 days. Morris Water Maze was used to study the spatial learning and memory in rats and the change in Escape transfer latency (ETL) was recorded to see the effect of drugs on it. Data analyzed by ANOVA followed by Dunnett's post-hoc test.

Results: Twenty one days drug treatment significantly increased the ETL in rats treated with Topiramate ($p=0.0001$) and combination of Levetiracetam and Topiramate ($p<0.0001$) from their baseline values. At the same time, there was significant reduction in the time spent in target quadrant in Topiramate group ($p= 0.033$) and the combination group of Topiramate + Levetiracetam ($p=0.026$). No significant change was observed in the other groups when tested for both these parameters.

Conclusion: Topiramate causes impairment of spatial memory in healthy rats after 21 days exposure and its combination with Levetiracetam could not overcome this cognitive deficit.

Keywords: Neuropathic pain, Non-epileptic, Spatial memory

INTRODUCTION

Many antiepileptic drugs (Carbamazepine, Phenytoin, Valproate, Lamotrigine, Gabapentin, Pregabalin and Topiramate) are used for non-epileptic indications like bipolar disorder, anxiety, neuropathic pain, prophylaxis of migraine, fibromyalgia, etc. Antiepileptic drugs (AEDs) act by multiple mechanisms like modulation of γ-aminobutyric acid (GABA) or glutamatergic neurotransmission, and alteration of voltage-gated ion channels or intracellular signaling pathways. These mechanisms may also explain the efficacy of AEDs in the treatment of bipolar disorder and neuropathic pain [1].

Cognitive problems like attentional and memory difficulties [2] as well as lower IQ in children with Phenobarbitone; decline in concentration, memory, visuomotor functions and mental speed with Phenytoin [3]; poor verbal fluency, detrimental effects on memory [4] and worse arithmetic performance with Carbamazepine; attentional dysfunction with valproic acid [5]; deterioration in verbal memory with Tiagabine [6]; impaired concentration, cognitive dulling, psychomotor slowing, language and comprehension problems, detrimental effects on short-term memory and working memory [7] with Topiramate are known in epileptic situations. But in other situations such problems are not yet reported to that extent. This study was conducted to evaluate the effects of AEDs on working memory as measured by delayed spatial alternation behaviour in non-epileptic rats and have found that AEDs can disrupt working memory, but there are differences among AEDs in the magnitude of the disruption which do not appear to be correlated with their mechanism of action [8].

Levetiracetam is a newer antiepileptic drug which is approved as an add-on therapy for partial onset seizures. Its off-label uses are absence seizures, migraine, neuropathic pain [9], bipolar disorder [10] and anxiety disorders. Structurally related to a class of drugs "nootropic agents", it has been reported in many preclinical and clinical studies that long-term levetiracetam treatment alone or as add-on therapy does not interfere with cognitive function and improves quality of life [11,12]. Studies have even demonstrated that Levetiracetam might help in improving cognitive abilities like, visual short-term memory [13], working memory [14], motor functions, psychomotor speed and concentration [15].

When used alone for nerve pain and bipolar disorder, the therapy with such drugs may be prolonged and occurrence of cognitive problems is always a possibility [16,17]. Hence, their long term effect on cognition needs to be studied in a non-epileptic situation. As the experience goes, carbamazepine and topiramate have the most detrimental effects on cognitive functions, hence they were chosen to be studied in combination with levetiracetam and this study was planned to study the extent of cognitive impairment with these antiepileptic drugs in healthy rats and evaluate the effect of co-administration of levetiracetam with carbamazepine and topiramate in this scenario.

MATERIALS AND METHODS

The study protocol was approved by Institutional Animal Ethics Committee {BVDUMC/443/2012-13 (proposal no.10)}. Wistar albino rats of either sex weighing 150-200 grams were used. The animals were maintained in a temperature-controlled room (22 ± 2°C) on a 12-h light/dark cycle with food and water available ad libitum. The rats were randomly assigned to six experimental groups with 6 animals in each group. Group I - Normal saline (control group). Groups II, III & IV were experimental groups of single drugs- Levetiracetam (180mg/kg), Carbamazepine (50mg/kg) and Topiramate (20mg/kg) respectively. Groups V & VI were drug combination groups: V- Levetiracetam (180mg/kg) + Carbamazepine (50mg/kg) and VI- Levetiracetam (180mg/kg) + Topiramate (20mg/kg). All the drugs were given orally for 21 days.

Morris' water maze Study [18]: It is used to study behavioural differences in the animals. It is among the commonly used tasks in behavioural neuroscience for studying the psychological processes

and neural mechanisms of spatial learning and memory. Animals, usually rats or mice, are placed in a large circular pool of water and required to escape from water onto a hidden platform whose location can normally be identified only using spatial memory.

The Morris' water maze apparatus consists of a circular water tank of 1.83 meters in diameter, divided into four quadrants (Q1, Q2, Q3 & Q4). There was a 4inch x 4inch size escape platform submerged in one of the quadrant, which was called the target quadrant. The top surface of the platform was hidden approximately 1 cm below the surface of the water. The pool was filled with water at a temperature of 18-26° C to a depth of about 40 cm. Milk was added to the water just before the experiment to make water opaque. Permanently positioned distinctive objects were placed for facilitating spatial orientation of the animal. Positions of the cues were kept unchanged throughout the period of training. In this test, rats were learning to swim in a water tank to find an escape platform hidden under water. Learning was reflected on the shorter latencies to escape and the decrease on the length of the path to find the platform. The experiment was conducted in two parts, acquisition trail (training trial) and retrieval trial. In acquisition trail, each animal was subjected to four consecutive trials on each day with an interval of 5 minutes, during which the animal was allowed to escape on the hidden platform and remain there for 20 seconds. In case of the inability of the animal to locate the hidden platform within 90 seconds, it was gently guided by hand to the platform and allowed to remain there for 20 seconds. Escape latency time (ESL) to locate the hidden platform in water maze was noted as an index of acquisition and learning. ESL was calculated as time taken by the rat from being placed in the water maze up to the time when the animal reached the platform.

Each animal was subjected to training trials for four consecutive days, the starting position was changed with each exposure as mentioned below and target quadrant (Q4 in the present study) remained constant throughout the training period:-

Day 1	Q1	Q2	Q3	Q4
Day 2	Q2	Q3	Q4	Q1
Day 3	Q3	Q4	Q1	Q2
Day 4	Q4	Q1	Q2	Q3

The retrieval trial was conducted 24 hours after the last acquisition trial (day 5). In this trial, the platform was removed and each animal was allowed to explore the pool for 90 seconds. The time taken by the animal to reach the target quadrant and time spent in the target quadrant were measured. Greater latency to reach the target quadrant and less time spent in the target quadrant suggests memory impairment [18]. After noting down the baseline readings, the animals were given the study drugs orally for 21 days and the change in Escape latency time was recorded to see the effect of drugs on it.

STATISTICAL ANALYSIS

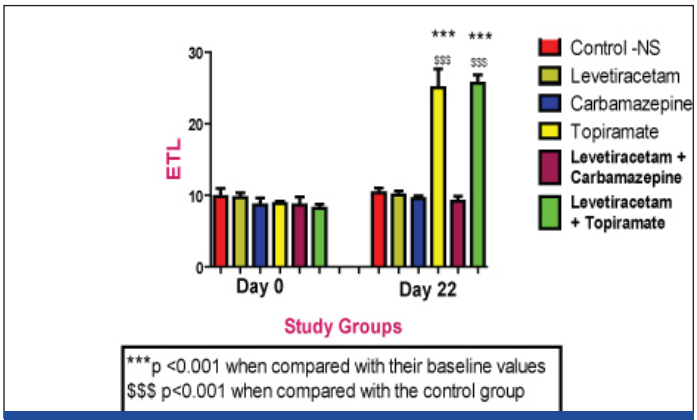
All the results are expressed as Mean ± S.E.M. Data was analyzed by one way analysis of variance (ANOVA) followed by Dunnett's post-hoc test in Graph Pad Prism version 5. $p < 0.05$ was considered as significant.

RESULTS

In the present study, we determined the effect of 21 day oral administration of Levetiracetam, Carbamazepine and Topiramate on the cognitive function of healthy rats. We also evaluated the effect of co-administration of levetiracetam with carbamazepine and topiramate in this setting.

When the extended transfer latency (ETL) of study animals was evaluated using Morris water maze, baseline values of all the study groups were comparable to each other. After 21 day drug treatment, significant increase in the ETL was observed in groups treated with Topiramate ($p=0.0001$) and combination of Levetiracetam + Topiramate ($p<0.0001$) from their baseline values. Similar results of increase in ETL were also noted in the same group animals (Group IV & VI) when compared with the control group treated with normal saline ($p=0.0003$ and $p<0.0001$ respectively). In the other treatment groups (Levetiracetam, Carbamazepine and Levetiracetam + Carbamazepine), there was no statistically significant difference between the ETL from its baseline to reading on the 22nd day [Table/Fig-1].

As seen in [Table/Fig-2], the time spent in target quadrant of Morris water maze was similar in all the study groups at baseline. After 21 days drug treatment, significant reduction in the time spent in target quadrant was found in Topiramate group ($p= 0.033$) and the combination group of Levetiracetam + Topiramate ($p=0.026$) from their baseline values. Control group and other drug treated groups (levetiracetam, carbamazepine and levetiracetam + carbamazepine) did not show significant change in this parameter and animals took nearly same time to reach the target quadrant on the 22nd day of the experiment as their baseline.



[Table/Fig-1]: ETL of study rats in Morris water maze

S. No.	Treatment groups	Time spent in Target quadrant (Day 0)	Time spent in Target quadrant (Day 22)
1.	Normal saline (Control)	24.33 ± 3.42	24.17 ± 3.27
2.	Levetiracetam	23.5 ± 3.06	24 ± 2.56
3.	Carbamazepine	25.33 ± 2.82	25.17 ± 2.6
4.	Topiramate	24.67 ± 2.53	14 ± 2.49 **
5.	Levetiracetam ± Carbamazepine	23.5 ± 2.84	23.17 ± 1.9
6.	Levetiracetam ± Topiramate	24.5 ± 1.62	13.82 ± 2.24 **

[Table/Fig-2]: Effect of study drugs on time spent in target quadrant of Morris water maze
**p< 0.01 when compared to the baseline values of the respective group

DISCUSSION

Cognition is a behavioural paradigm which incorporates attention, speed of intellectual functioning, linguistic and arithmetic skills, learning, memory, and co-ordination. Behavioural and cognitive dysfunctions are common problems in patients with epilepsy and these are of multi-factorial aetiology in which the underlying neuropathology of disease, psycho-social impacts and AEDs has been implicated.

Topiramate is a sulfamate-substituted monosaccharide that has multiple actions and is effective as adjunctive therapy for partial and primary generalized tonic-clonic seizures. As mentioned in the above section, topiramate is associated with a number of cognitive disabilities like somnolence and dizziness, emotional liability, impaired concentration and psychomotor slowing, as well as language problems with poorer performance on verbal tests [19 -21]. One of the most commonly cited reasons for discontinuation of topiramate therapy by patients, is the presence of cognitive side effects. In a multicenter, double-blind, randomized, prospective study [22] conducted in adults with partial seizures, authors have

found that the occurrence of cognitive adverse events and patient withdrawals from the trial related to cognitive decline were higher with topiramate than with lamotrigine.

Topiramate is being used for multiple indications in clinical practice but concern about its adverse effects in non-epileptic situations is not completely understood. As mentioned and experienced by researchers about the negative effect of antiepileptic topiramate on neuropsychological factors, even our study results indicate the same. In our study, it was found that Topiramate impaired the spatial memory of the animals in the Morris water maze model which is depicted by increased escape latency time (ETL) and decreased time spent in the target quadrant. Authors have studied that repeated administration of topiramate significantly reduces the activity of glutamine synthetase (GS) which results in a diminished capacity for the brain to metabolize, and thereby detoxify glutamate. The inhibition of GS may contribute to the toxicity of this compound. They also mention that drugs with an inhibitory action on the enzyme exhibit CNS-related toxicity, such as ataxia, sedation, dizziness and cognitive impairment [23].

Similar experience has been observed with low doses of Topiramate when used for other indications like migraine, bipolar disorder and obesity [24-26]. In most of these studies, a dose dependent effect on cognitive abilities had been observed. The studies done in epileptic situations also suggests that the deleterious effects of topiramate are dose related and reversible which need a close monitoring of the efficacy with titration of the dose of topiramate. As the experience goes with carbamazepine, variable results on cognitive parameters have been reported in epileptic situations with this drug [27-29]. The active metabolite, carbamazepine-10,11-epoxide has been found to be contributing to these cognitive effects of carbamazepine [30]. The effects of carbamazepine on cognition have also been related to dose, duration of treatment and polypharmacy. When used for other conditions, the experience of this adverse effect is quite less. The present study may add to this lack of information on cognitive effects of carbamazepine in non-epileptic situation. Results of our study revealed no significant effect of this drug on spatial memory, a subset of cognitive ability.

The third antiepileptic drug evaluated for its cognitive effects in the present study, levetiracetam, has a novel mechanism of action and binds to synaptic vesicle protein 2A (SV2A) that is believed to assist with the coordination of synaptic vesicle exocytosis and neurotransmitter release [31]. This drug is well-tolerated with a relatively low incidence of behavioural and cognitive adverse effects [32,33]. Some studies have even reported an improved cognitive functioning with levetiracetam either used alone or in combination. The cognitive abilities improved with levetiracetam and reported in studies range from visual short-term memory [8], working memory and motor functions [9], psychomotor speed and concentration [10] to fluid intelligence [34].

The mechanism responsible for benefit of levetiracetam in cognition is explained by the prolonged reduction of abnormal spike activity which ameliorates impairments in learning and memory and fully reverses deficits in synaptic transmission and plasticity in the hippocampus [35]. This advantage with levetiracetam has been experienced by authors in their studies with this drug alone or as an add-on drug with other antiepileptic [7,36]. Our experience in this study with levetiracetam reveals that there was neither cognitive impairment nor improvement when it was given alone. When administered as add-on drug with carbamazepine or topiramate, levetiracetam did not improve cognitive ability nor could it prevent spatial memory impairment with Topiramate.

Inspite of the fact that levetiracetam is capable of improving the cognitive areas like working memory and psychomotor skills which are specifically impaired by the deleterious effects of topiramate, no benefit was experienced in our animal model of spatial memory even in healthy rats. This raises a question on the memory improving ability of levetiracetam in normal or non-epileptic situations. Further animal studies using different cognition models and clinical trials are needed to obtain relevant data on the effect on levetiracetam as add-on drug on cognitive parameters in non-epileptic situations.

CONCLUSION

Topiramate showed impairment of spatial memory in healthy rats after 21 days exposure and its combination with levetiracetam could not overcome this cognitive deficit. Levetiracetam and carbamazepine had no significant effect on cognition in this model, alone or in combination.

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