Original Article

Effect of lamotrigine, levetiracetam and phenytoin on learning and memory in albino rats

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ABSTRACT

Background: *Epilepsy is one of the most prevalent non-communicable neurologic diseases leading to significant disability and mortality. Complaints of impaired learning and memory are common in patients of epilepsy. Anti-epileptic drugs (AEDs) may further enhance this impairment. So the present study was carried out on albino rats to evaluate the effect of AEDs on learning and memory.*

Objective: *To assess the effect of lamotrigine, levetiracetam and phenytoin on learning and memory in albino rats.*

Material and Methods: *Albino rats of about 150 -200 gm of either sex were treated with drugs for 15 days and assessed for effect on learning behavior and again treated for next 15 days after which they were assessed for retention behavior (memory) on Morris water maze and Elevated plus maze. The data was statistically analyzed by applying Mann- Whitney test.*

Result: *Phenytoin and lamotrigine caused significant impairment of learning whereas levetiracetam had no statistically significant effect on learning. Phenytoin also caused significant impairment of memory whereas lamotrigine and levetiracetam did not cause statistically significant impairment of memory.*

Conclusion: *Learning was impaired by phenytoin and lamotrigine but not by levetiracetam which has novel mechanism of action. Phenytoin resulted in memory impairment on Morris water maze but no impairment on elevated plus maze and no other drug caused this effect.*

KEY WORDS: *Anti epileptic drugs, learning, memory, elevated plus maze, morris water maze*

Introduction

Epilepsy is one of the most prevalent non-communicable neurologic diseases leading to significant disability and mortality. [1] Complaints of impaired learning and memory are common in patients of epilepsy. Anti – epileptic drugs (AEDs) may further enhance this impairment. [2] So study of effect of AEDs on learning and memory is of particular importance.

Learning and memory are most important part of cognition. So impairment of learning and memory function can also be stated in terms of impaired cognition. All commonly used AEDs have some effect on cognitive function. The effect become substantial when crucial functions are involved. For example learning in children, driving ability in adults or when already-vulnerable functions are involved, such as memory in elderly patients.

Many new AEDs like oxcarbazepine, vigabatrin, lamotrigine, zonisamide, gabapentin, tiagabine, topiramate and levetiracetam have been introduced into clinical practice within the last decade. Most of these new drugs are as effective as the old AEDs (phenytoin, Phenobarbital). In

general newer AEDs seem to be better tolerated than the older drugs. The new AEDs might produce less impairment of cognitive functions but this aspect has not been systematically studied[3]. So there is a need to systematically assess the role of AEDs in development of cognitive impairment in epileptic patient. [3] The study comparing the effect of newer and relatively older drug will be of particular importance since it will reveal the relative difference of impairment caused by these drugs, so it will be very helpful in choosing the drug for the treatment.

It is important to understand that whether the impairment is caused by the disease itself or by the AEDs so that further preventive measures will be taken. But it is difficult to distinguish between impairment associated with the disorder and those attributable to the drugs used for treatment. In an attempt to isolate the cognitive deficits associated with the drugs themselves, this study investigates the effects of antiepileptic compounds on learning and memory in normal animals.

Barbiturates and benzodiazepines are known to impair cognition in healthy volunteers as well as patients with epilepsy. Carbamazepine, phenytoin, and valproate have been reported to adversely affect cognition to a similar extent, although the magnitude of the effects of these three drugs appears to be less than that of barbiturates and benzodiazepines. [4] The effects of newer AEDs are less well studied, but several reports have suggested that newer AEDs such as lamotrigine and levetiracetam may have fewer effects on cognition than do older drugs. [5-7]

This study compared cognitive impairment caused by one of the more commonly used anti epileptic drug, phenytoin which is effective against both partial seizures and generalized tonic clonic seizures with that of lamotrigine which is currently considered first line drug against both partial as well as generalized tonic clonic seizure, and levetiracetam the newest drug in the group used as an adjunct in partial seizures. [8]

This study was undertaken to assess the effect of anti – epileptic drugs on learning and memory in albino rats with the aims and objective of the study as following

1. To observe the effect on learning and memory of three drugs i.e. lamotrigine, levetiracetam and phenytoin.
2. To compare the effect on memory and learning of newer anti epileptic drugs i.e. lamotrigine & levetiracetam with older anti epileptic drug i.e. phenytoin.

Material and Methods

This study was done in the Department of Pharmacology and Therapeutics, Rajendra Institute of Medical Sciences, Ranchi. Approval of the Institutional Animal Ethics Committee (IAEC), RIMS, Ranchi was taken prior to the start of this study.

24 healthy albino rats of about 150 - 200 gm in weight were divided in four groups (control, phenytoin, lamotrigine & levetiracetam) of six animals each. Each animal was placed in separate cages. Standard laboratory conditions of temperature, humidity and feeding were maintained.

The doses of the drug were determined on the basis of ratio of surface area of rat and man (0.018). Thus doses in rat were calculated by multiplying the absolute human dose by a factor of 0.018. Then each animal was weighed one week before the experiment and absolute dose

was calculated according to their body weight.

Separate suspension of each drugs were prepared by mixing the drug in normal saline with gum acacia. All the three drugs were made to strength of 10mg/ml by mixing 100mg of the respective drugs with 10ml of normal saline. Only freshly prepared drugs were used each day. The control group received 0.5 ml of normal saline.

Administration

All the drugs were given orally with a bent stainless steel feeding needle specially made for rats. The lumen size of the feeding tube is 18G.

Duration

All the rats received respective treatment for the period of 15 days after which they were examined for their learning behavior on Morris water maze and elevated plus maze for five consecutive days (day1 to day 5). The rats again received all the respective treatment for next 15 days after which they were examined again (on day 20) on Morris water maze and elevated plus maze to evaluate retention of past event (memory).

Apparatus

Morris water maze (MWM)

The Morris water maze is one of the most widely used tasks in behavioral neuroscience for studying the psychological processes and neural mechanisms of spatial learning and memory. It has gained a position at the very core of contemporary neuroscience research. [9]

It Consist of a large circular tank of diameter 1.8- 2.0 m and 0.4-0.5m in height. The pool is filled with water and rendered opaque by addition of non toxic color. The tank is marked off into four quadrants, i.e. North, South, East & West. An escape platform of 13 cm square size with heavy base is placed in middle of any fixed quadrant. To hide the platform water is added to a level 2 cm above the platform. The room should have potential extra maze cues that help to navigate the tank.

Procedure

Rats were placed in the water at a designated starting location and the time to find the hidden platform from the starting point is defined as “Escape Latency”. Each rat was tested for four trials/day with inter trial period of two minute during which they were placed in their home cage. Selection criteria – The rats for water maze were preselected. Rats that do not go to the visible platform on training and testing trials in the allotted time of 120 seconds were excluded from the study. Also, animals that refuse to search for the hidden platform during training and float on the water were removed from the study.

Elevated Plus Maze (EPM)

It is a validated method to test parameters of learning and memory [10, 11] to evaluate spatial long term memory in rodents. Introduced by Pellow (1985) in rats based on apparent natural aversion of rodents to open and high spaces. Based on this etoh et al. has demonstrated that transfer latency was markedly shortened if the animal had previously experienced entering in closed arm, and this shortening has been related to memory process. Apparatus for rat consist of two open (50 x 10 cm) and two enclosed arms (50x10x40 cm). The entire maze is elevated to a height of 50 cm.

Procedure

The rats were placed at the edge of open arm with facing away from the closed arm. Transfer latency is the elapsed time between the time the animal is placed in the open arm and the time in which all its leg have crossed a line marking initiation of

closed arms. Each rat was examined twice/day on successive open arm and time to reach in closed arm was noted by stopwatch. Selection criteria- The animals were preselected and those who don't cross the line in 120s were excluded from the experiment. The data was collected and effects on transfer latency time after administration of drug were compared with each drugs and control.

Data entry was done on MS EXCEL and 'SPSS version 17' software was used for data analysis. Mann- Whitney test was used to compare the effect of the drugs on different groups. Mann-Whitney test is a non parametric test used to compare two independent groups of sampled data. A non parametric test was applied in this study as the sample size in this study is less than 30 as well as the data is not normally distributed since a cut off time of 120s has been set prior in selection criteria. $P<0.05$ was considered significant.

Results

Phenytoin versus Others

Phenytoin causes significant impairment in learning compared to the control as well as with levetiracetam group on both MWM & EPM (Table 2, 3; day1-5). It also causes significant impairment of memory on MWM (Table2, day 20). But there was no statistically significant impairment as compared to lamotrigine group (Table 2, 3).

Levetiracetam versus Others

Levetiracetam does not cause any significant impairment in learning as compared to control group on MWM (Table 2; day1-5). But it was found to impair learning on day 4 in elevated plus maze (Table3). It has no significant effect on memory on both MWM as well as EPM (Table 2, 3; day20).

Lamotrigine versus Others

Lamotrigine causes significant impairment in learning compared to the control on both MWM and EPM (Table2, 3; day1-5). Lamotrigine causes significant impairment as compared to levetiracetam group in MWM (Table2) but no statistically significant impairment in EPM (Table3). There is no significant difference between the impairment caused by lamotrigine group compared to phenytoin group except on day 5 in EPM (Table3).

But lamotrigine fails to show any statistically significant impairment in memory as compared to control on both MWM (Table1) as well as EPM (Table2).

Table 1: Escape & Transfer latency time (i.e the time to reach the target) on Morris water maze and elevated plus maze respectively

	Morris water maze(Escape latency time)				Elevated plus maze(Transfer latency time)			
	Control	Phenytoin	levetiracetam	lamotrigine	Control	Phenytoin	levetiracetam	lamotrigine
Day1	34.80±5.81	41.08±4.75	37.87±4.36	40.58±3.25	70.66±14.56	89.25±14.60	75.83±12.01	94.91±5.13
Day2	21.33±7.22	34.79±5.81	21.33±7.22	32.08±3.28	29.58±3.73	44.33±13.67	63.00±13.11	47.75±9.44
Day3	14.95±1.76	30.75±1.95	19.04±1.27	30.75±1.95	17.25±1.31	24.25±3.76	39.08±8.23	41.50±7.27
Day4	12.66±1.35	24.25±6.92	11.91±1.05	20.87±4.09	10.91±1.06	28.75±5.57	28.08±9.97	28.33±9.57
Day5	7.70±0.92	21.33±7.22	7.95±0.44	19.16±5.09	10.25±0.85	23.83±6.98	14.91±4.28	11.08±0.52
Day20	15.00±1.95	29.03±1.95	19.70±1.72	22.07±2.43	80.83±13.71	89.58±14.37	76.00±12.02	94.08±5.75

Each value represents the Mean±SEM of six animals after taking averages of four trials /day

Table 2: Significant levels (p value) obtained after day wise comparison of change in escape latency time (drug versus control and drug versus drug) on MWM

	Day1	Day2	Day3	Day4	Day5	Day20
Phenytoin Vs control	0.093	0.041*	0.002*	0.132	0.002*	0.004*
Lamotrigine Vs Control	0.093	0.065	0.002*	0.132	0.022*	0.093
Levetiracetam Vs Control	0.485	1.0	0.093	0.699	0.937	0.132
Phenytoin Vs Lamotrigine	0.485	0.818	1.000	0.937	0.937	0.132
Phenytoin Vs levetiracetam	0.699	0.041*	0.002*	0.093	0.002*	0.015*
Lamotrigine Vs levetiracetam	0.394	0.065	0.002*	0.093	0.002*	0.699

* indicates significant values (P<0.05) using Mann-Whitney test

Table 3: Significant levels (p value) obtained after day wise comparison of change in transfer latency time (drug versus control and drug versus drug) on EPM

	Day1	Day2	Day3	Day4	Day5	Day20
Phenytoin Vs control	0.240	0.589	0.180	0.004*	0.016*	0.485
Lamotrigine Vs Control	0.394	0.132	0.065	0.015*	0.937	0.818
Levetiracetam Vs Control	0.818	0.180	0.065	0.041*	0.485	0.937
Phenytoin Vs Lamotrigine	0.937	0.699	0.093	0.394	0.041*	0.937
Phenytoin Vs levetiracetam	0.699	0.041	0.002*	0.093	0.002*	0.485
Lamotrigine Vs levetiracetam	0.310	0.395	0.699	0.818	0.485	0.310

* indicates significant values (P<0.05)

Discussion

Determining the effects of AEDs on cognitive function in nonepileptic subjects, both human and laboratory animals, permits assessment of the effects of AEDs on cognition without the added complexities of the disease state. Cognition in individuals with epilepsy may be

influenced by several factors, including basic neuropathology and the frequency and severity of seizures. The present study compared the effects of three AEDs on learning in nonepileptic rats. Phenytoin is one of the most widely prescribed anti seizure compounds. It was the first anti epileptic compound which have non sedative action at ordinary doses and that led to its extensive use. Recent evidence suggests that despite the success in controlling these types of seizures, phenytoin has profoundly negative effects on learning and memory processes. [12, 13] The results in this study are consistent with these findings. Thus, these data suggest that there is an apparent learning and memory deficits associated with maintenance on phenytoin. There is a need to carefully investigate when prescribing this drug, particularly in vulnerable population like children, where long-term developmental considerations need to be accounted for as well [14] and older adults particularly for their driving ability.

Little is known as yet about the effects of levetiracetam on cognitive performance. However, in healthy volunteers, levetiracetam had less effect on performance than did carbamazepine or oxcarbazepine. [15] In patients with epilepsy, add-on therapy with levetiracetam was reported not to produce significant changes in cognitive performance. [16] In fact, Cramer et al. [17] reported that add-on therapy with levetiracetam improved performance on the Cognitive Functioning. The findings in these studies are consistent with above studies as levetiracetam did not cause a statistically significant impairment learning and memory in comparison to the control group. Lamotrigine has been reported not to produce statistically significant cognitive effects in healthy volunteers or in patients with epilepsy. [18] The present result differs from these previous studies. The present study indicated that lamotrigine also causes statistically significant impairment of learning compared to control. There is no significant difference between the impairment caused lamotrigine and phenytoin. The cause of this difference in result from previous literature is not known. But since both phenytoin and lamotrigine mainly acts by blocking Na^+ channels and thus preventing repetitive firing in neurons, the result obtained in this study can be explained on the basis of their similar mechanism of action. Thus it suggests that further research in this field is warranted to establish the fact.

This work showing impact of drugs phenytoin, lamotrigine and levetiracetam on learning and memory is important especially when the drugs are used in:

a. Children in whom process of learning and memory is in process of development.
b. Old aged persons where memory is impaired due to senile degeneration, whether these drugs can precipitate memory loss or senile dementia is still a controversy
c. In neurological condition presenting with borderline mental retardation-whether these drugs will further accentuate the deficit.

The purpose of the present study was to evaluate the effects of AEDs on learning and memory as measured by Morris water maze and elevated plus maze task in nonepileptic rats. The major finding of the present study is that

1. Learning was impaired by the Na^+ channel blockers i.e. phenytoin and lamotrigine but not by levetiracetam which has novel mechanism of action.

2. The effect of these drugs on memory is not very clear from the present study as no drug has shown any statistically significant impairment on memory except for phenytoin which has shown impairment only on Morris water maze but no impairment on elevated plus maze.

In brief, it can be suggested that though the conclusion mentioned as above are in partial correlation of older work, further research in this line is needed in large number of subjects to stamp the effect of these drugs on learning and memory.

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