A STUDY ON EFFECT OF TRAZADONE, AMOXAPINE AND VENLAFAXINE ON MES (MAXIMAL ELECTROSHOCK) INDUCED SEIZURES IN ALBINO RATS

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Summary

This study demonstrated that Trazadone (50 mg/kg) and venlafaxine showed significant protection against MES induced seizures in albino rats. Amoxapine and Trazadone (100 mg/kg) failed to show any protection against MES induced seizures. Combination of low dose Trazadone and subtherapeutic dose of Phenytoin showed significant protection against MES induced seizures. A total of 42 animals (N=42) with a weight ranging from 200g-250g were used in this study with 6 (n=6) animals in each of the 7 groups. Group 1 received normal saline1ml/kg intraperitoneal (i.p), Group 2 received Phenytoin (25mg/kg, i.p), Group 3 received tablet. Amoxapine (18mg/kg, oral). Group 4 and 5 received tab. Trazadone (50mg/kg, 100mg/kg, oral respectively), Group 6 received inj.Venlafaxine (22.5mg/kg, i.p), and Group 7 received combination of sub therapeutic dose of Phenytoin (6 mg/kg, i.p) and Trazadone (50mg/kg, oral). The animals will receive maximal electro shock stimulation through trans-auricular electrodes with a current of 150 mA for 0.2 seconds. Abolition of Hind limb tonic extension (HLTE) and duration of HLTE, time taken for regaining righting reflex were observed. Complete absence of HLTE and significant decrease in time taken for regaining righting reflex were taken as protection. Our study proved that Venlafaxine and trazodone(50mg/kg) had anticonvulsant activity.

Key words: Maximal electroshock, Trazodone, Amoxapine, Venlafaxine, Anticonvulsant

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Introduction

Epilepsy is one of the most common neurological disorders. Worldwide, the prevalence is estimated to be 0.5 – 1%, and there is a life time incidence of 1 – 3%.[1] It has important medical, social and psychological consequences[1]. Despite the introduction of several new therapeutic options in the 1990s, a significant fraction of the patients with epilepsy continue to live with uncontrolled seizures.[1]

Mood disorders in patients with epilepsy remain unrecognized and are very often incorrectly treated. Precise diagnosis and effective therapy are very important because of a high suicide rate. Approximately 30% to 70% epileptic patients have the incidence of depressive disorders in their lifetime. Depression may have stronger influence on the quality of life than do the signs of epilepsy[2]. In clinical settings, 43% of patients with epilepsy and major depressive disorder (MDD) and 68% with minor depressive disorder were untreated, and 38% of those who had a history of lifetime episodes of MDD had never received antidepressant treatment.[3]

There are serious consequences of the lack of recognition and treatment of mood disorder in people with epilepsy resulting in increased morbidity and mortality. The incidence of suicide in people with epilepsy is, at least, five times higher than in the general population[4].

Of the anti depressants used in epileptic patients, amoxapine has shown greatest propensity to induce convulsions specially at higher doses[5]. And few studies has shown that trazadone, fluoxamine exhibit lowest seizure risk[6]. Some antidepressants (AD) may display anticonvulsant effects, especially when administered at low doses in experimental models of epilepsy and clinical settings.

The available data suggest, however, that ADs could have both proconvulsant and anticonvulsant effects and that drug dosage is the most important factor in determining the direction of action[7].

Trazodone is a first atypical antidepressant and selectively but less efficiently blocks 5HT uptake and has a weak $5HT_2$ antagonistic action. The later may contribute to its antidepressant effect. Amoxapine is tetracyclic compound is unusual in that it blocks dopamine D_2 receptors in addition to inhibiting NA reuptake. Venlafaxine is a bicyclic selective serotonin and noradrenaline reuptake inhibitor which increases the concentration of these amines in brain[8].

The potential importance of Selective Serotonin Reuptake Inhibitors (SSRI's) in modulating aspects of brain electrical activity has been described recently. Fluoxetine is known to act through a novel modulatory site on the GABA-A receptors, which mediate most fast inhibitory neurotransmission in the mammalian brain. This has shown significant anticonvulsant activity[9].

It is probable that drugs increasing serotonergic transmission have lower convulsant liability or, even, are more anticonvulsant than other Antidepressants (Ads)[10].

So this study has been undertaken to compare the effect of trazodone, amoxapine, and venlafaxine in MES (maximal electroshock) induced seizure in albino rats, to observe the effect of combination of Trazadone and sub-therapeutic dose of Phenytoin on MES induced seizures and to observe the dose dependent effect of Trazadone on experimental induced seizures.

Materials and Methods

Animals

The study was done in male Sprague dawley rats (150–200 g). The rats were housed under standard conditions with food and water ad libitum, which was bred in the central animal house J.J.M. Medical College, Davangere. They were used to induce convulsions by Maximal electroshock method.

A total of 42 animals with a weight ranging from 150g-200g were used in this study with 6 animals in each of the 7 groups. Group 1(control) received normal saline i.p (intraperitoneal), Group 2 received Phenytoin (standard, 25mg/kg, i.p), Group 3 received Amoxapine (18mg/kg, oral). Group 4 and 5 received Trazadone (50mg/kg, 100mg/kg, oral respectively), Group 6 received Venlafaxine (22.5mg/kg, i.p), and Group 7 received combination of sub therapeutic dose of Phenytoin (6 mg/kg, i.p) and Trazadone (50mg/kg, oral). The animals will receive maximal electro shock stimulation through trans-auricular electrodes with a current of 150 mA for 0.2 seconds after 30 min after i.p injection and 60 min after the oral dose. All the test animals were subjected to further experiment of this study after 24hrs to avoid any possible "Kindling" effect. Hind limb tonic extension (HLTE) (absent or present), duration of HLTE and time taken for regaining righting reflex was taken as parameters.

Drugs and chemicals: The standard drug phenytoin, tablet trazodone, amoxapine, obtained from institutional pharmacy, injection venlafaxine obtained from pharmacy shop were used in this study.

Maximal Electroshock-Induced Seizures

MESs were induced using a electroconvulsometer with a current of 150-mA intensity for 0.2-second duration via earclip electrodes. In rats maximal seizures consisted of initial tonic flexion, tonic hindlimb tonic extension (THLE), and terminal clonus. The endpoint of efficacy was taken as the inhibition of THLE. This was defined as protection and expressed as a percentage. Duration of HLTE and time taken for regaining righting reflex were also taken as secondary parameters.

Statistical Analysis:

Results are presented as Mean ± SEM. One way ANOVA was used for multiple comparisons followed by Tukey's post hoc test for comparison between groups. For all the tests a 'P' value of 0.05 or less was considered for statistical significance. Statistical analysis was done by using Graphpad prism version 5 statistical software.

ANOVA (Analysis of variance):

In statistics, analysis of variance is a collection of statistical models and their associated procedures, in which the observed variance is partitioned into components due to different explanatory variables. In its simplest form ANOVA gives a statistical test of whether the means of several groups are all equal and therefore generalizes Student's two sample t-test to more than two groups.

Post-hoc test:

Post-hoc tests (or post-hoc comparison tests) are used at the second stage of the analysis of variance (ANOVA) if the null hypothesis is rejected. The question of interest at this stage is which groups significantly differ from others in respect to the mean. In the present study Tukey's test was used for post-hoc comparison.

Results

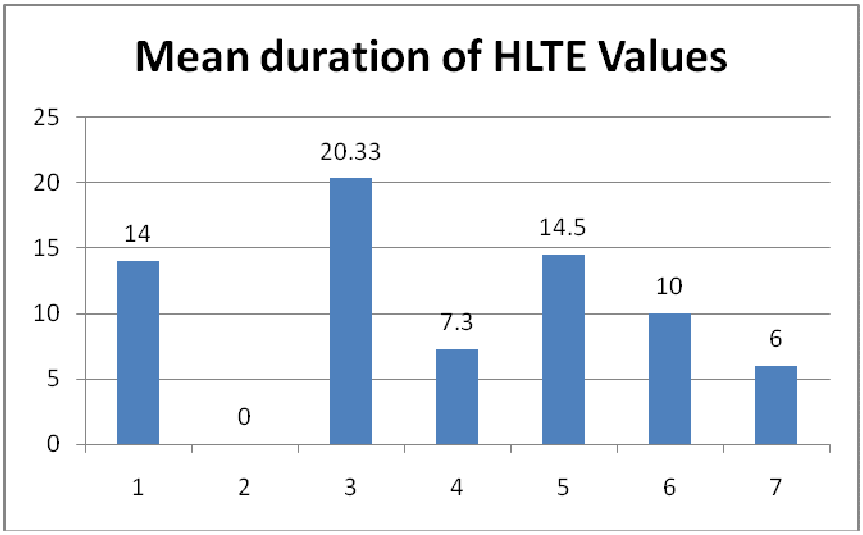
The present study revealed that Phenytoin sodium showed 100% protection against MES as compared to control. Trazodone (50mg/kg, oral) showed 33% protection and significant decrease in time taken for regaining righting reflex as compared to normal saline where as trazodone (100mg/kg, oral) and amoxapine (18mg/kg, oral) showed no protection. Nearly 84 % of animals in Venlafaxine (22.5mg/kg, i.p) group were protected against MES induced seizures. Phenytoin in subtherapeutic dose (did not show any protection, 6 mg/kg, i.p) did not show any protection but combination of Trazadone (50mg/kg, oral) and subtherapeutic dose of phenytoin (6 mg/kg, i.p) showed nearly 84% protection against MES induced seizures. These results showed antiepileptic effect was seen with low dose of trazodone and it also potentiated the anticonvulsant effect of subtherapeutic dose of phenytoin. But trazodone 100mg/kg did not show any anticonvulsant activity. Our study also showed that venlafaxine was effective in preventing MES induced seizures in rats and it also proved more effective than trazodone 50mg/kg group and its effect was similar to subtherapeutic dose of phenytoin and trazodone group.(table 1)

In case of venalafaxine group, the time duration of HLTE was significantly less compared to trazodone 50mg, trazodone 100mg/kg and amoxapine 18mg/kg groups ($P < 0.001$). In trazodone 50mg/kg group, subtherapeutic dose of phenytoin and trazodone, duration of HLTE was also decreased when compared to trazodone 100mg/kg and amoxapine 18mg/kg groups. So this proved that venlafaxine and trazodone 50mg/kg are effective in MES induced seizures (table 1, graph 1). The time taken for regaining righting reflex was also less in trazodone 50 mg/kg group, venlafaxine group and combination group when compared to control and amoxapine group.

Table 1: Showing percentage of protection with duration of HLTE and time for regaining righting reflex.

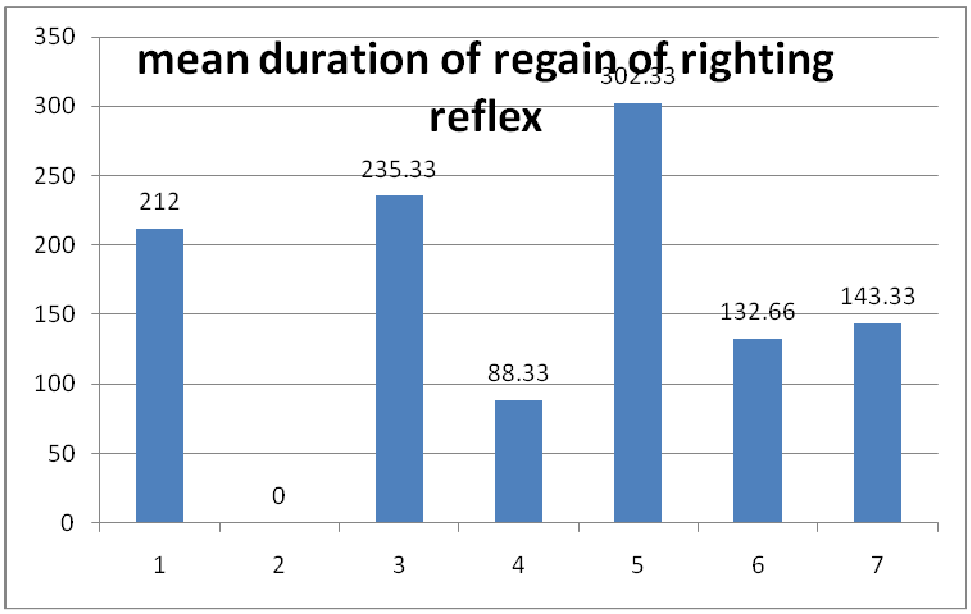
Drug	% of protection (%)	Mean duration of HLTE (sec)	Mean duration to regain righting reflex (sec)
Normal saline	0	14+/- 3.03	212.0
phenytoin	100	0	0
Amoxapine	0	20.33+/- 2.58	235.33
Trazodone 50mg/kg	33	7.33+/-5.8	88.33
Trazodone 100mg/kg	0	14.5+/- 2.7	302.33
Venlafaxine	84	1.0+/-2.4	132.66
Subtherapeutic phenytoin + trazodone	84	1.6+/-4.0	143.33

Graph 1 : Mean duration of HLTE



Note: 1= normal saline, 2= phenytoin, 3= amoxapine, 4= trazodone 50, 5= trazodone 100, 6= venlafaxine, 7= sub phenytoin+ trazodone.

Graph 2 : Mean duration for regaining righting reflex



Note: 1= normal saline, 2= phenytoin, 3= amoxapine, 4= trazodone 50, 5= trazodone 100, 6= venlafaxine, 7= sub phenytoin+ trazodone.

Discussion

This study demonstrated that Trazadone (low dose) and venlafaxine (22.5 mg/kg) showed significant protection against MES induced seizures in albino rats. Amoxapine and Trazadone (high dose) failed to show any protection against MES induced seizures. Combination of low dose Trazadone and sub therapeutic dose of Phenytoin showed significant protection against MES induced seizures.

The bioamine theory of depression implicates that there is decreased level of 5-HT and norepinephrine. Most of the clinically used drugs in depression act by inhibiting reuptake of 5-HT or norepinephrine or both. Fluoxetine is a known SSRI, by increasing serotonin concentration in brain it acts on novel modulatory site on the GABA-A receptors, mediate most fast inhibitory neurotransmission in the mammalian brain. This has shown significant anticonvulsant activity[9]. Another study shows that the possible relationship between brain biogenic amines i.e. endogenous norepinephrine (NE) and serotonin, but not dopamine depletion attenuated audiogenic seizures [11]. It was suggested that both 5 HT and NE play a major role in inhibiting seizure activity in the brain[12] and such type of depletion of biogenic amines results in "release" of inhibitory influence on nerve transmission. [13]

The $5HT_{2A/2C}$ receptors are predominantly located in cortex, neocortex, hippocampus and caudate nucleus. The precise roles in the CNS remain unclear. But in rodent agonist's activity over $5HT_2$ receptor evoked in motor behaviors [14], Trazodone is a first atypical antidepressant and selectively but less efficiently blocks 5HT uptake and has a weak $5HT_2$ antagonistic action. The latter may contribute to its antidepressant effect. In our study trazodone (50mg/kg) showed 33% protection in MES induced seizures. Its effect significantly

increased when it combined with subtherapeutic dose of Phenytoin. The anticonvulsant action of Trazodone could be due to activation of $5HT_{2\ A/\ 2C}$ receptors in the cortex of brain, which enhanced the 5-HT and norepinephrine by blocking its reuptake. Another possibility could be, stimulation of $5HT_{2A/2C}$ sub type receptors which are located on pyriform cortex GABAergic interneurons in brain, and their activation enhances GABA release which decreases firing of involved neurons[15]. The mechanism of lack of effect of higher dose of trazodone in MES induced seizures is no clear.

Venlafaxine is an atypical antidepressant used to treat various neurological disorder which mainly act by blocking the reuptake of serotonin and norepinephrine (NA), at low doses it acts mainly on serotonergic mechanism and at moderate to higher doses it acts on serotonin, NA and dopaminergic pathway.[16] Venlafaxine at lower doses by increasing serotonin which acts on GABA-A receptors potentiate the inhibitory action and this may shows its anticonvulsant activity. In this study 84% of the animals in venlafaxine group were protected from MES seizures, the possible mechanism of action is by potentiating GABA action by increased 5-HT.

But the Amoxapine is a tetracyclic compound, unusual in that it blocks dopamine D_2 receptors in addition to inhibiting NA reuptake, did not show any protection against the MES induced seizures in this study, probably due to amoxapine not having any effect on serotonergic pathway in brain. From the therapeutic point, side effects of antipsychotics which block D_2 receptors include epilepsy as side effect, so may be blockade of D_2 receptor by amoxapine may be the other reason behind the lack of protection against MES seizures.

To conclude Mood disorders are an important problem in people with epilepsy because they are often undiagnosed and incorrectly treated. Moreover, depressed epileptic patients generally display more severe seizure activity and greater problems with seizure recovery. Precise diagnosis and effective therapy are very important because of high suicide rate amongst patients with epilepsy. When considering treatment, one must take into account the positive effects of AEDs, the use of safer ADs at appropriate dosages, the potential for drug interactions, and the importance of adequate maintenance therapy.

Since depression is very common in the epileptic population, further human research is needed in this field, especially to clarify the effects of trazodone and venlafaxine on seizure threshold from an experimental point of view. Finally, it is important to identify clearer and safer guidelines for therapeutic management of patients suffering from epilepsy and depression.

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