EFFECT OF ESCITALOPRAM AND VENLAFAXINE ON SEIZURE SCORE AND LIPID PEROXIDATION IN MICE RECEIVING CARBAMAZEPINE ANTIEPILEPTIC THERAPY

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ABSTRACT

The frequent co-existence of depression in epileptic patients raises the issue of simultaneous use of antidepressants along with anti-epileptic drugs in management of such cases. However, it is necessary to evaluate the safety of these antiepileptic/antidepressant drug combinations. The present study investigates the effect of either antidepressant; escitalopram (selective serotonin reuptake inhibitor, SSRI) or venlafaxine (serotonin/noradrenaline reuptake inhibitor, SNRI), administered alone or in combination, with the conventional antiepileptic drug carbamazepine on chemo-convulsions induced by picrotoxin. In addition, the effect of both antidepressants on lipid peroxidation, as an assumable cause of neuro-degeneration in epilepsy, was studied in a model of chronic restraint in mice. The results show enhancement of seizure severity with significant increase in lipid peroxidation upon escitalopram treatment whether alone or in combination with carbamazepine. On the other hand, venlafaxine, administered alone or in combination with carbamazepine, provided significant protection against picrotoxin-induced convulsions as well as lipid peroxidation favoring its application in management of epilepsy-depression co-morbidities.

Keywords: Escitalopram – Venlafaxine – Seizure .

INTRODUCTION

Epilepsy is one of the most common neurological disorders characterized by recurring excessive neuronal discharge, exhibited by transient episodes of motor, sensory, or psychic dysfunction, with or without unconsciousness or convulsive movements. In addition epilepsy may be associated with neuro-degeneration presumably due to abnormal lipid peroxidation (Turkdogan et al., 2002; Hamed & Abdellah, 2004). On the other hand, co-morbid depression is common in patients with epilepsy. A review of available studies (Hermann et al., 2000) suggested a high prevalence of mood (affective) disorders especially major depression (8%-48%) followed by anxiety (5%-32%) in patients with epilepsy. Although the mechanisms underlying epilepsy-depression relationship have not been clearly identified, yet depression in epileptic cases is multifaceted with many interacting neurobiological and psychosocial determinants. These include clinical features of epilepsy (seizure frequency, type, foci, or lateralization of foci) and neuro-chemical or iatrogenic mechanisms (Harden, 2002; Ahern et al., 2005).

The high prevalence of depression in epileptic patients may necessitate the addition of an antidepressant agent in therapy. However, the use antidepressant drugs in epileptics has been a matter of debate for clinicians because of reports that these drugs may have frank convulsant or pro-convulsant effects that increase seizure incidence (Pruerter and Norra, 2005). This might happen due to modulation of pre- and/or post-synaptic receptor function and rate of release of neurotransmitters as γ-amino butyric acid, noradrenaline, dopamine or serotonin (Buckley & McManus, 2002; Montgomery, 2005). So, it is important to recognize and assess possible implications of antiepileptic/antidepressant drug combinations in management of epileptic cases complicated by depression.

Venlafaxine (VENLA) and escitalopram (ESC) are considered among the most commonly used antidepressant drugs. Venlafaxine, a hydroxyl-cyclo-alkyl-phenyl-ethylamine derivative, is a bicyclic antidepressant which is structurally and pharmacologically related to the analgesic tramadol, but not to any of the conventional antidepressant drugs including tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors or reversible inhibitors of monoamine oxidase such as moelobemide. Venlafaxine has been termed a serotonin/noradrenaline reuptake inhibitor (SNRI) (Muth et al., 1986). On the other hand, escitalopram is the S-enantiomer of citalopram and both of them are SSRIs and have significant antidepressant activity (Ceglia et al., 2004).

The rational of this study is to 1) evaluate neuropharmacological interactions of the widely prescribed anti-
epileptic drug carbamazepine (CBZ) with the antidepressants venlafaxine and escitalopram in management of chemically-induced seizures in mice, and 2) study the effect of these drug combinations on lipid peroxidation in frontal cortex in the same animal models.

MATERIALS AND METHODS

Drugs and Chemicals

Picrotoxin (Sigma Chemicals Co., USA), carbamazepine (Tegritol; Novartis Egypt Ltd.), escitalopram (Cipralex; Lundbeck, Denmark), venlafaxine (Effexor; Wyeth-Ayerst, USA).

Animals

Albino mice (20-25 g) were divided into six groups, 12 mice each. They were housed in cages with a natural light-dark cycle and fed on a standard pellet diet and water ad libitum.

Picrotoxin-induced convulsions

All mice were given a single subcutaneous dose (3.5 mg/kg b.wt.) of picrotoxin (PTX) either in absence of any previous treatment (control group) or following administration of a single dose of the test drug(s) (treated groups). Accordingly, the study comprised the following six groups (Grs):

Gr. I: Control group receiving neither antiepileptic nor antidepressant treatment.

Gr. II: Carbamazepine (CBZ)-treated group: received CBZ (50 mg/kg b.wt./day).

Gr. III: Escitalopram (ESC)-treated group: received ESC (2.5 mg/kg b.wt./day).

Gr. IV: Venlafaxine (VENLA)-treated group: receiving VENLA (8 mg/kg b.wt./day).

Gr. V: CBZ/ESC-treated group: given CBZ and ESC treatment in doses of 50 mg/kg b.wt. po and 2.5 mg/kg b.wt. po respectively, and

Gr.VI: CBZ/VENLA-treated group: given CBZ (50 mg/kg b.wt./day) plus VENLA (8 mg/kg b.wt./day).

In treated groups, PTX was injected after a suitable latency corresponding to the time expected to reach a peak effect following administration of the respective test drug(s). The drug latency was estimated as follows: CBZ: 4 hours (Sluzewska & Chodera, 1992); ESC: 5 hours (Montgomery et al., 2001); VENLA: 2.4 hours (DeVane, 1994). Immediately after administration of picrotoxin, the animal was observed for 30 minutes. The onset of convulsive behavior as well as nature and severity of convulsions were carefully recorded using the scoring system 1-7 as follows: hyper-locomotion or piloerection (Erection of the skin hair) –1; stunning (immobile) or catatonic posture (assuming a fixed posture and inability to move) – 2; clonic body tremors (a series of involuntary muscular contractions due to sudden stretching of the muscle) –3; prolonged clonic tremors –4; tonic forelimb convulsions followed by clonus –5; repetitive tonic (prolonged muscular contraction) forelimb convulsions followed by clonus –6; tonic extension of both forelimbs and hindlimbs followed by clonus –7. A mean cumulative score was calculated for each treatment group for comparisons and statistical analysis. At the end of the PTX study for each group, animals were returned to their cages to continue with the chronic restraint stress study.

Chronic restraint stress procedure

Each mouse of the respective group was placed in a wire mesh restrainer 6 hours daily for five weeks. At the end of the restraint period, mice were moved to their cages. Mice of the control group (Gr I) received no treatment during the 5-weeks period, while mice of treated groups received treatment with corresponding drug(s) during the 5 weeks as follows:

Gr. I: Control group receiving neither antiepileptic nor antidepressant treatment Gr. II: CBZ-treated group: received CBZ (50 mg/kg b.wt./day).

Gr. III: ESC-treated group: was administered ESC (2.5 mg/kg b.wt./day).

Gr. IV: VENLA-treated group: receiving VENLA (8 mg/kg b.wt./day).

Gr. V: CBZ/ESC-treated group: given CBZ and ESC treatment in doses of 50 mg/kg b.wt. /day and 2.5 mg/kg b.wt/day, respectively, and

Gr.VI: CBZ/VENLA-treated group: given CBZ (50 mg/kg b.wt./day) plus VENLA (8 mg/kg b.wt./day).

Measurement of frontal cortex thiobarbituric acid-reactive substances (TBARS) as a marker of lipid peroxidation:

At the end of the 5 week restraint stress, frontal cortex was excised out of brain, rinsed with cold 0.14 M NaCl and homogenized in 25% ice cold 50 mM Tris-HCl buffer, pH 7.4 (Benjamin et al., 1978). One hundred and fifty microliter of the tissue supernatant of samples was diluted to 500 µl with deionized water. 250 µl of 1.34% thiobarbituric acid was added to all the tubes followed by addition of equal volume of 40% trichloroacetic acid. The mixture was shaken and incubated for 30 min in a boiling water bath. Tubes were allowed to cool to room temperature and the absorbance was read at 532 nm using zero concentration as blank (Gutteridge and Quinlan, 1983).

Protein determination:

The total protein content of frontal cortex homogenate was determined according to the method of Bradford, (1976).

Statistical analysis:

The results were presented as medians with 25 and 75 percentiles for seizure score and mean ± standard deviation (SD) for seizure onset and cortical lipid peroxidation. Data were analyzed using one-way analysis of variance.
Sahar Kamal (2007) Effect of Escitalopram and Venlafaxine on Seizure Score and Lipid Peroxidation in Mice

RESULTS

I. Effect of different drug treatment regimens on PTX-induced convulsions

Single CBZ treatment significantly ($P < 0.05$) delayed the onset and reduced the severity of PTX-induced convulsions compared to the control group. Treatment with ESC whether alone or in combination with carbamazepine significantly ($P < 0.05$) prolonged the onset latency of convulsions compared to the control group; however it was significantly ($P < 0.05$) less potent in this regard than single CBZ treatment. In the meantime, neither single ESC treatment nor ESC/CBZ combination decreased severity of convulsions. On the other hand, treatment with VENLA alone significantly ($P < 0.05$) delayed the onset and reduced the severity of convulsions compared to the control group. However, it was significantly ($P < 0.05$) less potent than single CBZ treatment with respect to prolonging onset latency or reducing severity of convulsions. Treatment with VENLA/CBZ combination significantly ($P < 0.05$) delayed the onset and reduced the severity score of convulsions in comparison with all other groups (Table 1).

II. Effect of different drugs and drug combinations on TBARS in nmol/mg tissue protein in frontal cortex of mice exposed to acute restraint model

Single CBZ therapy significantly ($P < 0.05$) lowered TBARS level compared to the control group. Administration of ESC alone or in combination with CBZ significantly ($P < 0.05$) elevated TBARS level in comparison with all other groups. On the other hand, single VENLA therapy as well as VENLA/CBZ combination significantly ($P < 0.05$) lowered TBARS level if compared to control, ESC alone and ESC with CBZ groups (Figure 1).

### Table (1): Modulation of onset and severity of picrotoxin (PTX)-induced convulsions in mice treated with either antidepressants, venlafaxine (VENLA) or escitalopram (ESC). The antidepressants were given either alone or in combination with the conventional antiepileptic drug carbamazepine (CBZ). Treatment with ESC alone or in combination with CBZ significantly ($P < 0.05$) delayed onset of convulsions compared to control group but failed to reduce their severity score. On the other hand, single treatment with VENLA significantly ($P < 0.05$) delayed onset of convulsions compared to control group, and when combined with CBZ it further significantly ($P < 0.05$) reduced the convulsion severity score compared to all other groups.

* $P < 0.05$ compared to control group, * $P < 0.05$ compared to CBZ group.

<table>
<thead>
<tr>
<th>Gr</th>
<th>Treatment</th>
<th>Dose (mg/kg) &amp; po route</th>
<th>Onset of convulsions (seconds)</th>
<th>Severity (score range:1-7)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25%</td>
</tr>
<tr>
<td>1</td>
<td>PTX</td>
<td>3.5</td>
<td>479.8 ± 29.22</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>CBZ</td>
<td>50</td>
<td>1503 ± 74.11*</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>ESC</td>
<td>2.5</td>
<td>716.3 ± 69.12*</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>VENLA</td>
<td>8</td>
<td>1028 ± 49.79*</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>CBZ+ESC</td>
<td>50 + 2.5</td>
<td>862.5 ± 34.03*</td>
<td>4.3</td>
</tr>
<tr>
<td>6</td>
<td>CBZ+VENLA</td>
<td>50 + 8</td>
<td>2494 ± 65.75**</td>
<td>1</td>
</tr>
</tbody>
</table>

* $P < 0.05$ compared to control group, * $P < 0.05$ compared to CBZ group.

Figure (1): Changes in cortical TBARS levels upon treatment with either antidepressant; escitalopram or venlafaxine given alone or in combination with the antiepileptic carbamazepine (CBZ). Administration of escitalopram alone or in combination with carbamazepine resulted in a significant ($P < 0.05$) rise in the cortical TBARS compared to different groups while venlafaxine, alone or in combination to carbamazepine, significantly reduced TBARS content when compared to different groups.

* $t = P < 0.05$ significant increase compared to all other groups.

** $t = P < 0.05$ significant decrease compared to all other groups.
DISCUSSION

The high incidence of psychiatric co-morbidities, especially depression and anxiety, seen in epileptic patients may require treatment of both disorders at the same time with a combination of antiepileptic and psychotropic drugs. Therefore the safety of these drug combinations should be evaluated in order to optimize the treatment of epilepsy. Interestingly, some antiepileptic drugs have a complex of proconvulsant and anticonvulsant activities (Dailey & Naritoku, 1996). On the other hand, although the risk of antidepressant-induced seizures, in general, is very low, most, if not all, antidepressant agents have a propensity to lower the seizure threshold, and most are associated with a clinical risk of seizures. The mechanism by which antidepressants cause seizures, however, is still not well established. Recently, it has been suggested that the proconvulsant effects of antidepressants may be attributed to their local anesthetic, anti-muscarinic or anti-histaminic properties (Montgomery, 2005).

Although many studies report that use of older antidepressants e.g., monoamine oxidase inhibitors and tricyclic antidepressants is frequently associated with the risk of seizures (Trimble, 1978; Edwards, 1979), yet the newer antidepressants e.g., selective serotonin reuptake inhibitors (SSRIs) and serotonin/norepinephrine reuptake inhibitors (SNRIs) are claimed to exhibit a better tolerability profile for most patients. The safety of such agents still should be thoroughly assessed for clinical application in epileptic cases.

There is a great debate about the use of SSRIs in management of depression complicating epileptic cases. Early introduced SSRIs reportedly might increase seizure frequency and intensity (Skowron & Stümel, 1992; Rosenstein et al., 1993). Surprisingly, though the reports about pro-convulsive actions of SSRIs are ever increasing, these agents are still widely used in epileptics to treat accompanying depression (Kanner et al., 2001). Intensive research has been carried out to produce safer SSRIs and recently Escitalopram has been introduced as a new SSRI that is claimed to have a lower incidence of seizure induction. Apart from SSRIs, another group namely SNRIs e.g., venlafaxine and milnacipran is considered among the most recent antidepressant groups introduced in clinical practice. Clinical trials claim high effectiveness of SNRIs in treatment of cases of depression and anxiety, however their safety in epileptic cases should be carefully evaluated (Montgomery, 2005).

The models of chemoconvulsion and chronic restraint used in this study evaluate the effect of either antidepressant; escitalopram or venlafaxine used alone or in combination with the highly effective conventionally used antiepileptic; carbamazepine on seizure threshold and also, on an oxidative stress marker namely “TBARS”. The results indicate that single carbamazepine use significantly attenuates PTX-induced convulsions and decreases TBARS levels. This may be attributed to blockade of sodium channels as well as the GABAergic and anti-glutamatergic effects of carbamazepine (Motohashi 1990; Kubova et al., 1993). Interestingly, the SSRI escitalopram, used alone or in combination with carbamazepine, showed significant proconvulsant properties. Compared to single carbamazepine-treated group, escitalopram decreased latency of PTX-induced convulsions and increased the intensity of seizures indicating that it lowered seizure threshold.

The oxidative stress and modulation of anti-oxidant enzyme activity may contribute to the central deleterious consequences of chronic stress (Sunanda et al., 2000; Grillo et al., 2003). One of the neuro-chemical complications associated with epilepsy is increased lipid peroxide levels, especially TBARS, in the brain (Sudha et al., 2001). Enhanced lipid peroxidation can induce seizure activity by direct activation of glutamine synthase, thereby permitting an abnormal buildup of the excitatory neurotransmitter glutamic acid (Scharfman et al., 2003). The significant rise in cortical TBARS with escitalopram administration whether alone or in combination with carbamazepine further supports pro-convulsant properties of escitalopram. Besides it suggests that escitalopram could diminish the anticonvulsant effect of carbamazepine.

In contrast to escitalopram, the SNRI venlafaxine significantly increased latency of PTX-induced convulsions especially when combined with carbamazepine. The onset of convulsions in venlafaxine/carbamazepine-treated group was almost doubled compared to single carbamazepine-treated group. On the other hand, venlafaxine, used alone or in combination with carbamazepine, significantly reduced severity of convulsions and lowered TBARS cortical level. So, venlafaxine appears superior to escitalopram as an antidepressant drug devoid of proconvulsant activity. Furthermore, the results suggest that venlafaxine may possess independent anticonvulsant activity making it an ideal candidate that does not interfere with the anti-epileptic effect of carbamazepine and may rather augment such effect.

The better profile of venlafaxine obtained in this study might be explained partly by its unique mechanism of action as a selective reuptake inhibitor of norepinephrine besides blockade of serotonin uptake. Norepinephrine neurotransmitter possesses both antidepressant and anticonvulsant activities (Szot et al., 1999). The present study could suggest that venlafaxine would be more efficacious in modulating both depression and convulsions and its use proves to be associated with a significant reduction of TBARS being one of the detrimental oxidative stress markers responsible for exaggerating seizure profile (Rizwan et al., 2004).

In conclusion, the management of epilepsy is a difficult task when associated with other neuro-psychiatric disorders. Therefore extreme caution should be exercised with respect to selection of the proper antidepressants to treat epilepsy-depression co-morbidities. The SSRI escitalopram is not recommended in management of such cases as it may exaggerate seizure frequency and intensity.
most likely as a result of increased brain levels of the oxidative stress marker TBARS. On the other hand, the SNRI venlafaxine, used alone or in combination with the antiepileptic carbamazepine, may serve as a proper candidate in management of epileptic cases complicated with depression. Beneficial characteristics of venlafaxine may be attributed to proper elevation of the central neurotransmitter norepinephrine. Besides it significantly reduces the brain oxidative stress marker TBARS in mice.

REFERENCES


