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Effects of Verapamil and Olanzapine in Terminating Pilocarpine-Induced Epileptic Seizures in Mice.

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Abstract

Background Epilepsy is one of the oldest known neurological conditions characterized by recurrent seizures.

Objective To explore the possible antiepileptic effect of both Verapamil and Olanzapine in pilocarpine epileptic model in mice.

Methods Fifty healthy male albino mice weighing between 30-35 gm were equally allocated into five groups (10 mice in each group) and were distributed into: normal group (without drug); distilled water group (0.1 ml); diazepam group (1mg/kg); verapamil group (20 mg/kg) and olanzapine group (10 mg/kg). All animals (except normal group) were injected with pilocarpine hydrochloride (350 mg/kg) to induce generalized tonic-clonic seizure 30 minutes after the tested drugs had been administrated. The mean onset of seizure were determined as well as the mean serum concentration of electrolytes, glutathione and malondialdehyde were measured after seizure had been induced.

Results Pilocarpine-induced seizure at approximately 7 minutes after injection. While both verapamil and olanzapine produced highly significant increase in mean onset of seizure 16±1.549 and 13.1±1.566 respectively as compared to D.W. group, also both drugs produced highly significant changes in mean serum concentration of electrolytes, glutathione and malondialdehyde.

Conclusion Verapamil and olanzapine had anticonvulsant activity when used at applied doses in the pilocarpine model of seizures in mice.

Key words Epilepsy, seizure, verapamil and olanzapine.

List of Abbreviation: 5-HT = 5-hydroxytryptamine, Bax = Bcl-2-associated X protein, Bcl-2 = B-cell lymphoma 2, Bcl-XL = B-cell lymphoma-extra-large, BDNF =brain-derived neurotrophic factor, GABA = gamma-aminobutyric acid, GSH = reduced glutathione, I.P = intraperitoneal, MDA = malondialdehyde, GSH = glutathione, SOD= superoxide dismutase.

Introduction

Epilepsy is one of the major neurological diseases [(1)], it is characterized by recurrent, unprovoked, paroxysmal episodes of brain dysfunction manifesting as a large number of clinical phenomena [(2)]. Epilepsy occurs due to many different cellular or biochemical changes such as alterations in ion channel function, neurotransmitter level (excitatory and inhibitory), neurotransmitter receptor function and energy metabolism, in addition to the body electrolytes, level of some trace elements, and membrane lipid peroxidation due to increase in free radicals or decrease in activities of antioxidant defense mechanisms all these may be causally involved in some forms of epilepsy and may increase the recurrence of seizures [(3)].

The pilocarpine provides a useful animal model for studying epilepsy. Generalized tonic-clonic convulsion induced by pilocarpine shows the

involvement of the cholinergic system in seizures and status epilepticus [4]. The activation of muscarinic receptors is the first step for seizure activity, while GABAergic and glutamatergic systems appear to mediate seizure propagation and/or maintenance in rodent epilepsy models [5].

Verapamil is an L-type calcium channel blocker of the phenylalkylamine class which has cardiac effects more than vascular smooth muscle effects. While olanzapine is an atypical antipsychotic, antimanic and mood stabilizing agent belongs to the thienobenzodiazepine class that demonstrates a broad pharmacological profile across a number of receptor systems [6], such as anticholinergic and antioxidant effects [7]. These effects of both drugs are useful in terminating of epilepsy. The current study was carried out to explore the possible antiepileptic effects of Verapamil and Olanzapine on pilocarpine induced seizures in mice.

Methods

This study was carried out on fifty healthy male albino mice weighing between 30-35 gm, they were supplied by animal house of Al-Nahrain College of Medicine and were housed under good conditions and fed standard oxoid palate with water ad libitum, and they were equally allocated into five groups (10 mice in each group):

- ***Group 1: (Normal group):*** This group served as normal control and takes no drug used to detect the normal values of serum electrolytes, glutathione (GSH) and malondialdehyde (MDA).
- ***Group 2: (Distilled water group):*** They were injected 0.1 ml of distilled water (I.P.) 30 mint. before pilocarpine injection, to induce epileptic seizures.
- ***Group 3: (Diazepam group):*** They were injected 1mg/kg of diazepam (I.P.) 30 mint. before pilocarpine injection. This group served as positive control and was used to compare onset of seizure only with tested groups.
- ***Group 4: (Verapamil group):*** They were injected verapamil 20 mg/kg (I.P.) 30 mint. before pilocarpine injection.
- ***Group 5: (Olanzapine group):*** They were injected olanzapine 10 mg/kg (I.P.) 30 mint. before pilocarpine injection.

After giving the pilocarpine, each mouse was carefully evaluated by detecting the parameters which includes the onset of the first seizure recorded by naked eyes. At the end of observations the blood samples were collected from survival mice for measuring other parameters which are the serum concentrations of Na^+, K^+ ,Ca^{2+}, GSH and MDA.

Statistical analysis was performed with the SPSS 19.0 statistical package for social sciences, data were expressed as mean ± Standard error of mean (S.E.M.), unpaired t-test at ($p \leq 0.01$) and ($p \leq 0.05$) for independent data was used [8].

Results

All the mice exhibited generalized limbic seizures after pilocarpine administration, at a latency of (7±0.394) minutes; besides, it caused highly significant reduction in mean serum Na^+, Ca^{2+} and GSH concentration, while it caused highly significant increase in mean serum concentration of K^+ and MDA when compared to that of normal group.

Diazepam caused highly significant increase in mean onset of seizure; also diazepam had no effect on other parameters when compared with D.W group.

Both verapamil and olanzapine showed highly significant increase, and highly significant reduction for olanzapine in mean onset of seizure when compared to that of both D.W. and diazepam groups respectively, this indicated that verapamil is more potent than olanzapine, also both drugs revealed highly significant increase in mean serum concentration of Na^+, Ca^{2+} and GSH with highly significant reduction and non-significant change in mean serum concentration of MDA and K^+ respectively when compared to that of D.W group(Table 1).

Table 1. Effect of Verapamil and Olanzapine on Onset of Seizure and Serum Na^+, K^+, Ca^{2+}, GSH and MDA Concentrations in Group I, II, and III in Pilocarpine-induced Seizure in Mice

Group	Seizure Onset (minute) M ± SEM	Concentration				
		Sodium mmol/l M ± SEM	Potassium mmol/l M ± SEM	Calcium mg/dl M ± SEM	GSH mmol/l M ± SEM	MDA mmol/l M ± SEM
I	0	144.8 ± 2.958	5.47 ± 0.212	8.5 ± 0.306	1.13 ± 0.063	5.02 ± 0.103
II	7 ± 0.394	133.2 ± 1.781a**	8.11 ± 0.502a**	7.03 ± 0.157a**	0.8 ± 0.052a**	10.66 ± 0.265a**
III	18.6 ± 0.858b**	134.5 ± 1.45	7.78 ± 0.54	7.35 ± 0.34	0.85 ± 0.05	10.5 ± 0.28
IV	16 ± 1.549b**	147.3 ± 3.464b**	8.38 ± 0.082 a**	10.12 ± 0.087a**, b**	1.605 ± 0.104a**,b**	5.8 ± 0.203a**, b**
V	13.1 ± 1.566b**,c**	151.9 ± 2.41b**	8.38 ± 0.44 a**	9 ± 0.522 b**	1.2 ± 0.051b**	8 ± 0.316a**, b**

*= $P \leq 0.05$, **= $P \leq 0.01$, a = as compared to group I, b= as compared to group II, c= as compared to group III), n = 10/group).

Discussion

Epilepsy is one of the most common neurological problems all over the world, being associated with paroxysmal discharge of cerebral neurons and is characterized by several symptoms including alterations of behaviors and consciousness sustained alteration in brain function [9].

The anticonvulsant activity of verapamil may be attributed to its ability in blocking the calcium channels and to prevent the increase in intracellular calcium which play important role in incidence of certain types of seizures [10,11].

In addition, calcium channel antagonist enhanced the anticonvulsive activity of carbamazepine, valproate and Phenobarbital against maximal electroshock-induced seizures in mice [12,13]. Verapamil is a known inhibitor of P-glycoprotein and may block P-glycoprotein–modulated efflux of antiepileptic drugs in the brain [14]. The antioxidant activity of verapamil may contribute to new neuroprotective activity by which verapamil inhibit free radical-induced damage to lipid constituents of the membrane, and by inhibition of cellular oxidative stress and reduction of MDA level and augmented the activity of antioxidant enzymes [15].

Furthermore, calcium channel antagonist have been found to inhibit the release of steroids and also block the inflow of sodium into detonated neuron as well as inhibit calcium-dependent glutamate channels, thereby inhibit the release of glutamate, an excitatory neurotransmitter which has been implicated in the pathogenesis of convulsive seizures from such neurons [16,17].

The increase in serum calcium and sodium concentration may be related to blocking calcium channel and antioxidant activity of verapamil.

Olanzapine has neuroprotective and cytoprotective effects mediated by the acceleration of metabolic processing of free radicals, since olanzapine increase SOD and decrease MDA level [7,18], also olanzapine increases the GABAergic neuroactive steroid allopregnanolone in rat cerebral cortex (which is a potent GABAA- receptor modulator with anxiolytic and anticonvulsant effects) [19].

Olanzapine attenuates cocaine neurotoxicity in a mouse model by blocking the dopamine, alpha adrenergic, 5HT-2 and 6 serotonin, muscarinic receptors and monoamine transporters [20].

The neuroprotective effects of olanzapine on the Okadaic acid (a selective and potent inhibitor of the serine/threonine phosphatases 1 and 2A) induced neurodegeneration and apoptosis in brain [21], which are mediated through the following mechanisms:

- Olanzapine upregulate the level of brain-derived neurotrophic factor (BDNF), an important neurotrophin mainly expressed and distributed in brain neurons that prevent cell degeneration [22].
- Olanzapine upregulate the level of Bcl-2 and modulate the Bcl-XL/Bax ratio in brain. Bcl-2, a neuroprotective protein that inhibit apoptosis [23].
- Olanzapine has antioxidant activity by increasing the gene expression of superoxide

dismutase (SOD1) in PC12 cells, and prevent cell death after serum withdrawal [18].

- Olanzapine can increase cell proliferation and neurogenesis in adult rat brain [24].

The increase in serum calcium and sodium concentration may be related to antioxidant, antimuscarinic activity and $Na^{+}K^{+}$ ATPase modulation of olanzapine. All the tested drugs had valid preventive effect against seizure induced by pilocarpine in mice when were compared to that of diazepam, verapamil had the most potent effect while olanzapine had the weakest effect, the latter can be used for schizophrenic patient with symptoms of epileptic seizures.

Finally the aim of present study to find a new drugs or combination of drugs which are more potent with less adverse effects to be used in epilepsy after confirmation with clinical trials.

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Author contributions

Uday is a researcher who has done the technique of this work and conducted the writing of manuscript. Dr. Faruk participated in supervision and in scientific review of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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