Original Article

Amlodipine potentiates the protective effect of zonisamide on pentylenetetrazol-induced kindling in mice

Abstract

Background: Zonisamide (ZON) and amlodipine (AML) were studied in different experimental models of epilepsy individually. However, combination of ZON and AML has not been explored yet. Hence, this study was aimed to evaluate the combination therapy of ZON and AML on pentylenetetrazol (PTZ)-induced kindling in mice. **Materials and Methods:** Swiss albino mice of either sex were randomly divided into five groups and each group containing 8 mice. Kindling was induced by PTZ (45 mg/kg/day on 8th, 10th and 12th and 75 mg/kg/day on 14th day). After treatment animals were observed for 30 min for behavioral analysis then animals were sacrificed and brain was taken out for biochemical analysis. **Results:** PTZ-significantly induced seizure was characterized by alteration in seizure score and latency as well as significantly increased in brain thiobarbituric acid reactive substance (TBARS) levels and significantly decreased in reduced glutathione, catalase (CAT) and superoxide dismutase (SOD). Treatment with ZON and AML significantly ($P < 0.05$, $P < 0.01$ and $P < 0.001$) resorted seizure score, latency, TBARS, reduced glutathione, CAT and SOD near to normal compared with pentylentetrazol treated rats. **Conclusion:** This study provides experimental evidence that treatment with both ZON and AML attenuated seizure and oxidative stress in PTZ-induced kindling mice.

Key words:
Amlodipine, kindling, oxidative stress, pentylenetetrazole, zonisamide

Introduction

Epilepsy is a neurological disorder characterized by recurrent spontaneous epileptic seizure associated with distinct neurological and behavior alterations.[1] About 50 million people are affected with epilepsy worldwide, of which up to 75% live in poor countries.[2] It is the second most common disorder of the central nervous system after stroke, with an incidence rate of 0.3–0.5% and is usually demanding long-term treatment.[3,4]

Pentylenetetrazol (PTZ), an epileptogenic drug, causes absent-type seizures at low doses, clonic seizures at moderate doses and tonic-clonic seizures at high doses.[5] The induction of convulsions by PTZ is attributed to repression of gamma-amino butyric acid (GABA) type A receptor gated Cl – channel, which attenuates the GABA dependent inhibition,[6] as well as augments production of free radicals.[7,8]

Zonisamide is a benzisoxazole derivative with a sulfonamide side chain and is structurally unrelated to other antiepileptic drugs (AEDs). It is a newer broad-spectrum AED and has been effective against refractive partial seizure.[9,10] Various preclinical studies reported that zonisamide (ZON) reduces calcium-dependent potassium evoked extracellular glutamate release in the hippocampus.[11] Furthermore, a number of studies have reported that ZON blocks low voltage-gated T-type calcium channel and enhance the level of GABA in the brain that may be associated with the antiseizure activity.[12,13] ZON has free radicals scavenging properties and thus, protects neuron by scavenging hydroxyl and nitric oxide radicals in a dose-dependent manner.[14,15]

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Calcium ion (Ca^{2+}) plays an essential role in the pathogenesis of epilepsy.[16] A decrease in the extracellular calcium concentration, followed by an increase in the intracellular calcium concentrations lead to the onset of seizure activity.[17] Amlodipine (AML), a calcium channel blocker, is used in the treatment of various cardiovascular diseases such as hypertension and angina pectoris.[4] However, a number of experimental studies reported that calcium channel inhibitors for examples AML, diltiazem and verapamil have the potential to reduce the incidence of seizures.[18] Some studies reported that dihydropyridines) have antiepileptic and antioxidant properties as well as augment the protective efficacy of some antiepileptic agents. Although, ZON and AML was studied in different experimental models of epilepsy individually. However, combination of ZON and AML has not been explored yet. Hence, this study was aimed to evaluate the combination therapy of ZON and AML on PTZ-induced kindling in mice.

Materials and Methods

Animals

Swiss albino mice of either sex (6–8 week old), weighing 25–30 g, were procured from the animal house facility, KIET School of Pharmacy, Ghaziabad. The animals were kept in polypropylene cages under standard laboratory conditions (12 h light and 12 h dark cycle), temperature (25 ± 2°C), and humidity (55 ± 5%) and had a free access to commercial rat pellet diet (Pranav Agro Industries, Delhi) and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethics Committee of KIET School of Pharmacy (Registration number 1099/07/CPCSEA dated 27.07.2007), Ghaziabad.

Drugs and chemicals

Zonisamide was obtained from Sun Pharmaceuticals Industries Ltd. (Synergy), Jammu and Kashmir, India; AML was obtained from Ranbaxy Laboratory, Gurgaon, India. All other chemicals used in the experiment were of analytical grade.

Experimental design

Swiss albino mice of either sex were randomly divided into five groups and each group containing 8 mice as follows:

Pentylenetetrazol control (i.e., Group I) rats treated with PTZ (45 mg/kg on 8^{th}, 10^{th} and 12^{th} and 75 mg/kg on 14^{th} day, intraperitoneal [i.p.]).

Zonisamide + PTZ (i.e., Group II) rats treated with ZON (50 mg/kg) 30 min before injection of pentylenetetrzole (45 mg/kg on 8^{th}, 10^{th}, 12^{th} and 75 mg/kg on 14^{th} day), for 14 days; AML (5 mg/kg) + ZON + PTZ (i.e., Group III) rats treated with ZON (50 mg/kg) and AML (5 mg/kg) 30 min before injection of pentylenetetrzole (45 mg/kg on 8^{th}, 10^{th}, 12^{th} and 75 mg/kg on 14^{th} day), for 14 days, respectively.

Amlodipine 10+ZON+ PTZ (i.e., Group IV) rats treated with ZON (50 mg/kg) and AML (10 mg/kg) 30 min before injection of pentylenetetrzole (45 mg/kg on 8^{th}, 10^{th}, 12^{th} and 75 mg/kg on 14^{th} day), for 14 days, respectively.

Amlodipine 20+ZON+ PTZ (i.e., Group V) rats treated with ZON (50 mg/kg) and AML (20 mg/kg) 30 min before injection of pentylenetetrzole (45 mg/kg on 8^{th}, 10^{th}, 12^{th} and 75 mg/kg on 14^{th} day), for 14 days, respectively.

Behavioral analysis

After each PTZ injection, the convulsive behavior was observed for 30 min. The resultant seizures were scored as follows: Stage 0 (no response); stage 1 (hyperactivity, restlessness and vibrissae twitching); stage 2 (head nodding, head clonus and myoclonic jerks); stage 3 (unilateral or bilateral limb clonus); stage 4 (forelimb clonic seizures); stage 5 (generalized clonic seizures with falling).[19]

Estimation of oxidative stress and antioxidant enzymes in brain

After treatment, the animals were anesthetized and then sacrifice, the brain was excised out, washed in ice-cold normal saline and weight. After that, the weight tissue from each group was homogenized w/v in ice-chilled phosphate buffer (50 mM, pH 7.4) or disodium ethylenediaminetetraacetic acid (0.02 M) or potassium chloride (0.15 M) and centrifuge at 10,000 g for 20 min in high speed cooling centrifuge. A clear supernatant was used for assaying different biochemical parameters according to the procedure.

Measurement of lipid peroxidation was carried out by determination of myocardial malondialdehyde content by the method of Ohkawa *et al*.[20] Antioxidant enzymes viz. glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were estimated in cardiac tissue as per standard protocol. GSH was estimated by the Sedlack and Lindsay method.[21] The activity of SOD was measured according to the method of Marklund and Marklund.[22] CAT activity was measured according to Clairborne.[23]

Statistical analysis

The data are expressed as mean ± standard error of the mean. All values were analyzed by one-way analysis of variance, followed by Turkey's test. Results were significant if $P < 0.05$.

Results

Effect on seizure score

All mice in each group survived without any complications at the end of the kindling period. In the PTZ group, repeated administration of a dose of PTZ (45 mg/kg on 8^{th}, 10^{th} and 12^{th} day and 75 mg/kg on 14^{th} day, i.p.) resulted

in increased convulsive activity leading to generalized clonic–tonic seizure. Figure 1 shows that preadministration of ZON (50 mg/kg) along with AML (5, 10, and 20 mg/kg, respectively) significantly inhibited seizure scores compared to PTZ. None of animal could achieve a score 5 with 4 injection of PTZ.

Effect on latency

Administration of PTZ significantly decreased latency of seizure at the dose 45 mg/kg on 8th, 10th and 12th day and 75 mg/kg on 14th day. Pretreatment with ZON (50 mg/kg) + AML (5, 10, and 20 mg/kg) significantly ($P < 0.01$ and $P < 0.001$) increased latency compared to PTZ. Increased in latency was observed at 20 mg/kg of AML along with ZON [Figure 2].

Effect on brain tissue thiobarbituric acid reactive substances

Table 1 shows brain levels of oxidative stress markers in the kindled and nonkindled mice. PTZ kindling produced a significant increase in the brain tissue thiobarbituric acid reactive substance (TBARS) level, an index of lipid peroxidation, as compared with PTZ control. Pretreatment with ZON + AML (5, 10, and 20 mg/kg, respectively) significantly ($P < 0.05$, $P < 0.01$, and $P < 0.001$) decreased TBARS levels compared to PTZ control group. AML decreased TBARS levels in dose-dependent manner.

Effect on brain tissue reduced glutathione

Table 1 shows that there was a significant decrease in the brain reduced GSH level in PTZ control group. Pretreatment with ZON + AML (5, 10, and 20 mg/kg, respectively) significantly ($P < 0.05$, $P < 0.01$, and $P < 0.001$) increased GSH levels compared to PTZ control group. This effect was observed highest in ZON + AML (20 mg/kg).

Effect on brain tissue catalase

Table 1 shows brain tissue CAT levels. There was significant decrease in CAT level in PTZ control group. Pretreatment with ZON + AML (5, 10, and 20 mg/kg, respectively) significantly ($P < 0.05$, $P < 0.01$, and $P < 0.001$) increased CAT levels compared to PTZ. This effect was observed highest in ZON + AML (20 mg/kg).

Effect on brain tissue superoxide dismutase

Table 1 shows brain tissue SOD levels. There was significant decrease in SOD level in PTZ control group. Pretreatment with ZON + AML (5, 10, and 20 mg/kg, respectively) significantly ($P < 0.05$, $P < 0.01$, and $P < 0.001$) increased SOD levels compared to PTZ

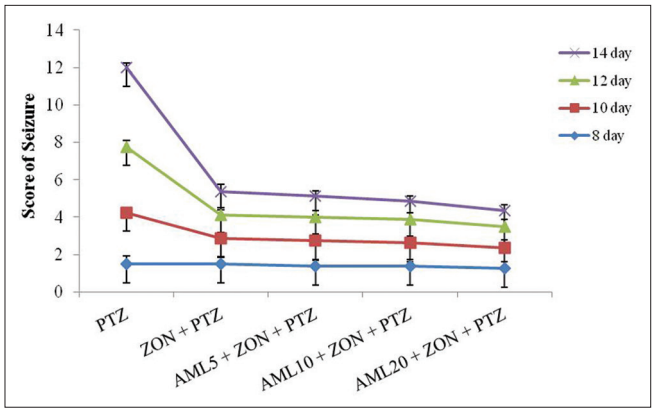

Figure 1: Effect of zonisamide and amlodipine on pentylenetetrazol-induced score of seizure

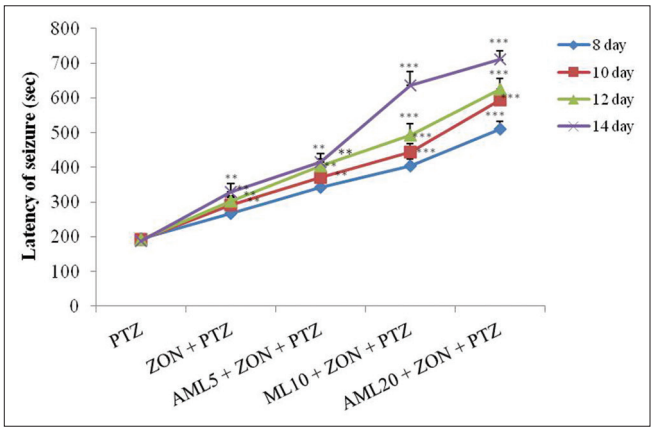

Figure 2: Effect of zonisamide and amlodipine on pentylenetetrazol-induced changes in latency of seizure

Table 1: Effect of ZON and AML on PTZ-induced changes in brain tissue TBARS, GSH, SOD, and CAT levels

Groups	TBARS (nmol MDA/mg protein)	GSH (μmol/mg protein)	SOD (IU/mg protein)	CAT (nmol H_2O_2-consumed/min/mg protein)
PTZ	7.23±0.25	2.13±0.16	1.13±0.33	24.99±1.28
ZON + PTZ	5.87±0.27*	3.23±0.25*	1.37±0.01*	36.01±0.37*
AML5 + ZON + PTZ	5.77±0.29*	3.34±0.20*	1.49±0.03*	37.43±1.76*
AML10 + ZON + PTZ	5.50±0.31**	3.57±0.26**	1.63±0.01**	48.58±1.63**
AML20 + ZON + PTZ	5.28±0.33***	3.92±0.15***	1.77±0.02***	55.75±1.88***

Data are expressed as mean ± SEM. *P<0.05; **P<0.01 and ***P<0.001 versus PTZ. TBARS – Thiobarbituric acid reactive substance; GSH – Reduced glutathione; SOD – Superoxide dismutase; PTZ – Pentylenetetrazol; ZON – Zonisamide (50 mg/kg); AML20 – Amlodipine (20 mg/kg); ZON + PTZ – Zonisamide (50 mg/kg) + pentylenetetrazol; AML5 + ZON + PTZ – Amlodipine (5 mg/kg) + zonisamide (50 mg/kg) + pentylenetetrazol; AML10 + ZON + PTZ – Amlodipine (10 mg/kg) + zonisamide (50 mg/kg) + pentylenetetrazol; AML20 + ZON + PTZ – Amlodipine (20 mg/kg) + zonisamide (50 mg/kg) + pentylenetetrazol; MDA – Malondialdehyde; SEM – Standard error mean; CAT – Catalase

control group. This effect was observed highest in ZON + AML (20 mg/kg).

Discussion

The results obtained in this study demonstrated that the combination therapy of ZON and AML exhibited significant anticonvulsive action against PTZ-induced kindling in mice. Epilepsy is associated with increased in neuronal excitation in brain as well as oxidative stress. Treatment with both ZON and AML decreased the seizure score and increased the latency as well as attenuated reactive oxygen species (ROS) generation. Thus, combination of these drugs ameliorated PTZ-induced kindling. Epileptic subject shows higher seizure score and lower latency period. In the present study, the PTZ treated animals showed a significant increase in score of seizure and latency. The results of the present study are in corroborates with the previous study.[24] Treatment with both ZON and AML significantly decreased seizure score and increased latency period. Combination therapy with ZON and AML showed protection against PTZ-induced convulsion in dose-dependent manner.

Various central nervous system disorders have been associated with the ROS generation, such as seizures.[25] ROS can damage essential cellular constituents such as lipids measured by TBARS.[26] In the present study, PTZ-induced seizure increases the levels of TBARS indicating that oxidative stress occurs as a consequence of seizures, thereby contributing to seizure-induced brain damage, which is similar with the previous study.[27] Animals treated with both ZON and AML significantly prevented PTZ-induced elevation in lipid peroxidation. It is also known that convulsions, followed by an increase in brain lipid peroxidation might be diminished by antioxidant enzymes.[28] Thus the results of present study suggest that, ZON and AML combination therapy have antiepileptic and antioxidant effects against PTZ-induced lipid peroxidation in brain tissue.

Furthermore, free radicals are normal products of cellular aerobic metabolism involved in the development of seizures.[29] Free radical scavenging enzymes such as GSH, CAT, and SOD are the first line cellular defense against oxidative stress, eliminating reactive oxygen radical such as superoxide (O_{2-}) and hydrogen peroxide (H_2O_2), and preventing the formation of more reactive hydroxyl radical (OH). This study is in corroborates with these findings where the decreased activities of GSH in PTZ-subjected mice were significantly increased on combined treatment with ZON and AML. This finding indicated that combined therapy of ZON and AML could improve the cellular antioxidative defense against oxidative stress.

Conclusion

We concluded that combined effect of ZON and AML attenuate PTZ induced kindling and oxidative stress. This effect may be due to a reduction in convulsion as well as a reduction in lipid peroxidation and augmentation of antioxidant enzyme activity. Thus, combination therapy of ZON and AML attenuate seizure and oxidative stress in mice. This could be due to increase in latency period and has strong antioxidant properties. However, further study is needed to unraveled the molecular mechanism.

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