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POSSIBLE ANTICONVULSANT EFFECT OF IVABRADINE IN KAINITE –INDUCED EPILEPSY IN RATS: AMELIORATION OF OXIDATIVE STRESS

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ABSTRACT

Background: Temporal lobe epilepsy is a longer neurological disorder in which patients suffer from spontaneous seizures. New treatments with novel mechanisms of action are needed to help those patients whose seizures are resistant to available drugs. In the present study, we evaluate the possible anticonvulsant effect of ivabradine, a hyperpolarization activated cyclic nucleotide-gated channel (HCN) blocker in kainite model of temporal lobe epilepsy in rat. In addition, evaluate the oxidative stress and nitric oxide alternation Material and Methods: adult male albino rats were divided into three equal groups: control group (CN), kainic acid (KA) induced epileptic non- treated rats, epileptic rats treated with ivabradine (20mg/kg/day) one week before convulsant state with KA epileptic rats. The latency to and the duration of tonic-clonic seizures were recorded. Also, brain oxidative stress markers were measures (lipid peroxidation, and nitric oxide). Results: of the present work revealed that convulsive state induced by KA (10 mg/kg I.P) resulted in significant elevation ($p \leq 0.05$) of NOx (NO metabolites, NO^{2-} plus NO^{3-} as indices of NO generation), lipid peroxidation malondialdehyde in the KA–alone treated group compared with normal rats. Administration of ivabradine (20mg/kg I.P) resulted in significant reduction ($P \leq 0.05$) of duration of clonic seizures, cortical NOx and lipid peroxidation. On the other hand, ivabradine significantly increased latency of tonic-clonic seizures. Conclusion: The present data promising the anticonvulsant and potent antioxidant effects of ivabradine in epileptic animals.

KEYWORDS: ivabradine, kainite-induced epilepsy, oxidative stress, HCN, tonic-clonic seizures.

INTRODUCTION

Epilepsy and seizures disorders affect 50 million people around the world and contribute to morbidity and mortality.[1] The use of antiepileptic drugs is limited due to the vast array of adverse effects. Such as cognitive impairment, effective disorders and recurring seizures.[2] Hence, there is a need for the development of new antiepileptic drugs with fewer adverse effects and high efficacy.

Kainic acid (KA), a prototype chemoconvulsant, is commonly employed for inducing experimental seizures for the evaluation of antiepileptic agents[3] A part from accumulating evidence indicates that oxidative stress might be involved in KA-induced neurotoxicity in vivo[4,5,6] and in vitro.[5] Activation of KA receptors results in nitric oxide synthase (NOS) activation, free radical formation, mitochondrial dysfunction, result in inflammatory responses, cytokine expression and oxidative stress through reactive oxygen or nitrogen species.[7,8] All plays an important role in the neuronal death processes.[9,10,11]

In different types of neurons a voltage-activated Na^+/K^+ current called "Ih" has been identified. This current (Ih = hyperpolarization-activated depolarizing current) is activated by membrane hyperpolarization facilitated by cAMP.[12] The molecular basis of Ih current has been characterized by cloning a family of ionic channels known as HCN- hyperpolarization activated cyclic nucleotide-gated channels,[13,14,15,16] Four isoforms of HCN.[12,17] have been identified in many tissues, including cardiac and neuronal tissues.[12,18,19,20,21] .In neurons, HCN channels have many functions, such as regulation of cell excitability, synaptic transmission and generation of rhythmic activity. [22] There is accumulating evidence that the current is involved in epileptogenesis.[23,24]

Ivabradine (a hyperpolarization activated cyclic nucleotide-gated channel (HCN) blocker), is a pure heart rate lowering drug regulation of heart rate in the sino-atrial node.[25,26]

Moreover, the drug is considered to be the most specific blocker of Ih current without effects on T-type and L-type calcium channels and delayed outward potassium channels.[27] Electrophysiological experiments revealed that ivabradine blocks mouse HCN1 and human HCN4 channels, being an open-channel blocker of HCN4 and a closed-channel blocker of mHCN1 channels.[20] It has some beneficial effect on nociception, inflammation, psychosis and amnesia,[28,29,30] A recent study.[31] showed that ivabradine elevated the threshold for

electroconvulsions in mice and thus possesses anticonvulsant potency against electro-shock-induced tonic seizures in mice.

The present study aims to determine the possible anticonvulsant effect of ivabradine. Moreover, to evaluate its effect on nitric oxide alternation and lipid peroxidation in kainite model of epilepsy in rats.

MATERIAL AND METHODS

Animals used

Adult male albino rats, weighing 140±20 gm were used for the experiment brought from (Experimental Animal Breeding Farm, Helwan-Cairo). All animals were housed in controlled laboratory condition at 20 -25C in a 12h light/dark cycle and had free access to standard laboratory chow (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and water. They have acclimatized for one week and were caged (6/cage) in fully ventilated room (at room temperature) in pharmacology department, Benha Faculty of Medicine. All experimental protocols were approved by the committee of Benha University.

Design study

An experimental model of epilepsy was created by injection of kainic acid (10 mg/kg i.p., Sigma, Saint Louis, MI, USA) in rats.[32] Animals were observed for myoclonic jerk latency and the occurrence of generalized tonic seizures up to 3 hours after KA injection.[33] and rated as described previously.[3] A 5-stage rating scale based on behavioral changes was used; Rats without any obvious behavioral changes were rated as stage 0; rats exposing only wet dog shakes as stage 1; rats with chewing, head bobbing and forelimb cloni as stage 2; rats with generalized seizures and rearing as stage 3; rats with generalized seizures, rearing and falling (loss of postural tone) as stage 4, and rats that died during status epileptics were rated as stage 5. The ability of a drug to prevent the seizures or delay/ prolong the latency or onset of the tonic hind-limb extensions was considered as an indication of anticonvulsant activity. Epilepsy was induced in groups of rats.

Group (1): control group (n=12), this group did not receive any drug they were given equivalent amount of drug vehicle (saline) I.P.

Group (2): epileptic non treated rats (n=12), this group did not receive any drug they were given equivalent amount of drug vehicle (saline) I.P.

Group (3): epileptic rats treated with ivabradine (20mg/kg/day I.P) one week before injection with KA (10mg/kg I.P) (n=12).

The doses of the drugs used in the present work were according to previous pilot experiments Luszczki et al.[31] The onset and duration of clonic seizures were detected in each group and represented as mean±S.E.

At the end of the experiments, rats were decapitated, the skull was opened, cerebral cortex was dissected and its NO was estimated as nitrite (NO^{2-}) and nitrates (NO^{3-}) according to Hashiguchi et al. [34] and lipid peroxidation level malondialdehyde (MDA), an end-product of peroxidation of cell membrane lipids caused by oxygen-derived free radicals, is considered a reliable marker of oxidative stress was determined by measurement of the chromogen obtained from the reaction of malondialdehyde with 2-thiobarbituric acid, according to Roghani & Baluchnejadmojarad.[35] The MDA values are expressed as nanomole per mg of tissue protein (nmol/mg protein).

Statistical analysis

Results of the present work were presented as mean± S.E. P values were calculated by unpaired (t) test, $P \leq 0.05$ was taken as the limit of significance.

RESULTS

1- Effect of ivabradine against KA-induced seizures

The present study showed that, the seizures reached class four (three hours after kainic acid administration) in 70.1% (9 of 12) of rats treated with kainic acid according to Spark's standard classification. When ivabradine was administered at dose of 20mg/kg/day one week before KA-injection, the latency of myoclonic jerks increased significantly ($p<0.05$) as compared with KA. There were also a decrease in the incidences and duration of generalized tonic-clonic convulsions (table 1).

2- Effects of ivabradine on the brain lipid peroxidation and nitrite

Kainic acid injection produced significant elevation in the levels of brain malondialdehyde (MDA) (43.42±3.22 ng/mg protein), cortical nitrite (12.88± 0.76 nmol/mg protein) compared with ivabradine treated group (MDA, 27.34±0.65 ng/mg protein; nitrite, 7.34±0.62 nmol/mg protein). Pretreatment of epileptic rats with 20 mg/kg ivabradine significantly decreased MDA and nitrite levels compared to non-treated group (figure 1, 2).

Table(1). Effect of ivabradine (20mg/kg I.P) one week before KA injection against epilepsy-induced by KA (10mg/kg I.P) in male adult rats showing that ivabradine significantly increased latency (hour) and decreased duration measured in minute of clonic-tonic seizures (means ±S.E)

Groups	Latency (hour)	duration of clonic seziures (min.)
Control group	0	0
Epileptic -non treated	2.7±0.3	10.6±0.7*
Ivabradine treated	3.6±0.1	7.9±0.5*

*significant effect in compared with epileptic non treated group.

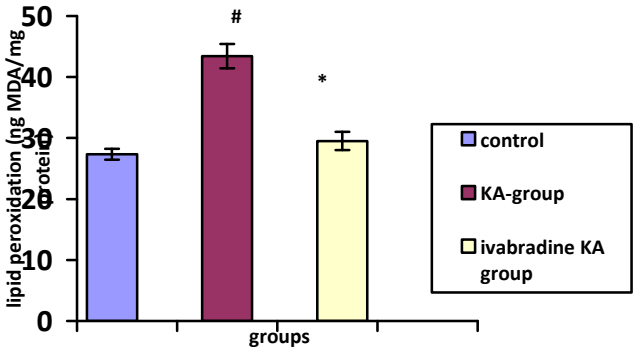

Figure (1): The effect of ivabradine on malondialdehyde (MDA) concentration in cortical homogenate from different groups. Pretreatment with ivabradine 20 mg/kg/day one week before kainate injection showed significant reduction of brain MDA in comparsion with non-treated group. * p<0.05. # Significant increase in brain lipid peroxidation (MDA) in comparison with normal control group.

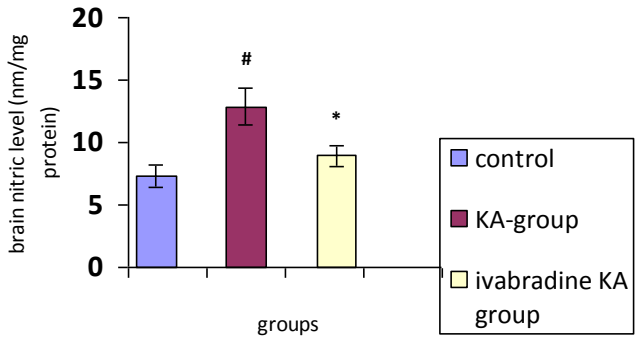

Figure (2): The effect of ivabradine on nitrite content in rat brain. The rats treated with ivabradine 20mg/kg one week before kainite injection showed significant decrease of nitrite content in comparison with non-treated group.* P<0.05. # Significant increase of nitric content in comparison with normal control group.

DISCUSSION

The present study aimed to explore the possible anticonvulsant effect of ivabradine and its effect on oxidative stress by analysis of brain nitric oxide and lipid peroxidation in kainic acid induced epilepsy in rats.

In this work, intraperitoneal injection of kanic acid was used to induce experimental seizures for the evaluation of antiepileptic agent.[3] The data of the present study showed that administration of kainic acid was followed with seizures, significantly increasing of MDA and nitrite level in rat brain. These results are in line with previous studies[36,37,6] which reported that the oxidative stress might be involved in KA-induced neurotoxicity in vivo and in vitro.[38] There are more and more reports suggesting that activation of KA receptors results in nitric oxide synthase (NOS) activation, free radical formation, mitochondrial dysfunction, result in inflammatory responses, cytokine expression and oxidative stress through reactive oxygen or nitrogen species.[7,8] All plays an important role in the neuronal death processes.[9,10,11]

It is a well-established fact that impairment of endogenous antioxidants plays a major role in the genesis as well as precipitation of seizure. Role of oxidative stress in CNS has been well demonstrated in several experimental models of epilepsy such as the amygdala kindling model, kainic acid model, PTZ kindling model.[39] Studies have been focusing on exploring whether prolonged seizure attack in animals results in increased reactive oxygen species production and whether oxidative injury plays an important role in seizure-induced brain damage.[40,39] Recent studies demonstrated that HCN channel activity is closely related to epileptogenesis. Moreover, kainic acid-induced seizure susceptibility is increased in HCN1-/- mice.[41] and HCN2-deficient mice exhibit spontaneous absence seizures.[42] HCN1-/- mice show a significantly higher number of negative resting membrane potentials and a significantly higher input resistance measured from responses to either negative or positive current steps.[43] As such, seizure susceptibility is increased in HCN1-/- mice.[41]

In this study, 7 days pretreatment of kainite rats with ivabradine (20mg/kg/i.p) as a HCN channel blocker improved spontaneous seizures, increased the latency of jerks, myoclonic seizure and shorten the duration of KA- induced convulsions suggesting its possible neuroprotective potential. Similarly, the current study confirms and extends previous findings[31] which demonstrated that ivabradine in dose dependently increase the threshold for maximal electroshock-induced tonic seizures in mice. It can supposed that ivabradin, due to

its HCN channel blocker properties, is able to support the ionic homeostasis in the brain, and therefore, the drug elevated the threshold for electroconvulsions in mice.

In addition, we have evaluated the anti-oxidant effect of ivabradin on brain nitric oxide content and lipid peroxidation which demonstrated significant improvement versus experimental non-treated KA induced epilepsy in rats. In accordance with previous reports[44,45,46] who reported that ivabradine directly ameliorates nitric oxide and lipid peroxidation induced renal inflammatory response in ischemic reperfusion injury in rats by inhibiting oxidative stress. This observations supported our result that the significant prolongation of latency onset and decreased duration of seizures may be due to antioxidant effect.

CONCLUSION

This study support the previous experimental findings that showed ivabradine associated with a prolongation of latency period of seizures by improving inflammatory status and oxidative stress reflecting the important role of HCN in epilepsy.

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