



Effect of Ivabradine against Pilocarpine induced Seizures in Rats

Osama Q. Fadheel¹, Faruk H. AL-Jawad¹, Waleed K. Abdulsahib², Haider F. Ghazi³

¹Pharmacology Department, College of Medicine, Al-Nahrain University, Baghdad, Iraq.

²Pharmacy College, Al- Farahidi University, Baghdad, Iraq.

³Microbiology Department, College of Medicine, Al-Nahrain University, Iraq.

*Corresponding author's E-mail: waleedkalel22@yahoo.com

Received: 16-08-2018; Revised: 22-09-2018; Accepted: 06-10-2018.

ABSTRACT

Epilepsy is a standout amongst the most widely recognized genuine mind issue. Study was performed to investigate the conceivable antiepileptic impact of Ivabradine against pilocarpine incited seizure in male rats. Four gatherings were utilized as a part of this examination. Rats of each gathering (aside from typical gathering) were infused intraperitoneal with pilocarpine hydrochloride (400 mg/kg) following 21 long periods of tried medications organization orally. The mean beginning and length of seizure, neuroprotective impact, NMDA receptor, Sodium diverts were estimated in all gatherings after writhing had been prompted. Ivabradine had a preventive and anticonvulsant impact against pilocarpine incited seizure in rats because of diminishing the beginning and seriousness of seizure.

Keywords: Epilepsy, Ivabradine, Neuroprotective effect and Pilocarpine hydrochloride.

INTRODUCTION

Epilepsy and seizures issue influence 50 million individuals around the globe and add to grimness and mortality¹. The utilization of antiepileptic drugs is constrained because of the immense range of unfriendly impacts. For example, subjective disability, compelling scatters and repeating seizures². Thus, there is a requirement for the improvement of new antiepileptic drugs with less antagonistic impacts and high adequacy. Ivabradine is a drug utilized for the symptomatic administration of stable heart-related chest agony and heart disappointment not completely overseen by beta blockers³. Ivabradine follows up on the I_f (f is for funny) particle current, which is very communicated in the sinoatrial hub. I_f is a mixed Na^+-K^+ inward current activated by hyperpolarization and modulated by the autonomic nervous system. It is a standout amongst the most critical ionic streams for directing pacemaker movement in the sinoatrial (SA) hub. Ivabradine specifically restrains the pacemaker I_f current in a measurements subordinate way. Obstructing this channel decreases cardiovascular pacemaker action, specifically moderating the heart rate and enabling more opportunity for blood to stream to the myocardium⁴.

MATERIALS AND METHODS

Forty male Wister rats (200– 300 g) were bought from the Animal house at the College of Medicine/AL-Nahrain University in Baghdad. The creatures were housed under temperature, moistness and light-controlled conditions. Every creature convention was affirmed by the Institutional Review Board at the College of Medicine/AL-Nahrain University. In addition, they were sustained

standard oxide sense of taste with water not indispensable.

Pilocarpine-incited status epilepticus

The Pilocarpine-incited status epilepticus techniques were executed as already depicted by⁵. Status epilepticus was characterized as a period of nonstop seizures that went on for no less than 5 min or seizures that repeated at short interims (<1 min) setting up a continuing epileptiform condition⁵. Every single ensuing investigation was performed in the intense period of pilocarpine-instigated epilepsy. Seizures (summed up limbic seizures with status epilepticus) were incited by a solitary intraperitoneal organization of pilocarpine hydrochloride 4% (400 mg/kg)⁷.

Pretreatment tests

Four groups of rats were utilized for the pretreatment tests:

Each group contain 10 rats were taken standard and Ivabradine for 21 days orally.

Group 1: (Normal group): This gathering not got any medication was filled in as typical control gathering to distinguish the ordinary qualities.

Group 2: (pilocarpine incited epilepsy bunch just): were taken pilocarpine infusion intraperitoneal (400mg/kg) considered as epileptic control.

Group 3: (Valproic group): They were taken 20 mg/kg twice day by day of sodium valproate orally before pilocarpine infusion. This group filled in as positive control to look at tried gatherings.



Group 4: (Ivabradine group): They were taken Ivabradine 1 mg/kg/day orally before pilocarpine infusion.

Parameters

In the wake of giving the pilocarpine, each rodent was precisely assessed by distinguishing the beginning of the primary seizure, term of seizure, repetitive of seizures and demise, recorded by exposed eyes, different parameters incorporate the sodium current through sodium channels, movement of NMDA receptors, Neuro N (measure of neural cell passing).

Immunohistochemistry

Guideline of the test

This strategy in view of the location of the antigen utilizing particular rodent antibodies that ties to particular focused on protein. The bound essential immune response at that point identified by optional neutralizer (hostile to rodent) conjugated with biotin, the auxiliary counter acting agent is then recognized by streptavidin conjugated with horse radish peroxidase polymer which catalyze the substrate H₂O₂ into free oxygen and water (H₂O). The free oxygen at that point oxidize 3, 3'-diaminobenzidine (DAB) into dim darker hasten. A positive response will have demonstrated as a darker shaded encourage at the antigen site counter recolored with hematoxylin and tried for immunoreactivity⁸.

Immunohistochemical Procedure

Pre-recoloring steps

These means incorporate planning of slides for recoloring steps this can be expert by the accompanying advances:

- A. Slide arrangement: Paraffin installed segments were cut into 5µm thickness, at that point the areas were conveyed by cement emphatically charged slides, segments were left to dry to encourage grip between the segment and the charged glass surface.
- B. Deparaffinization and rehydration: this progression include:
 1. Dewaxing of paraffin inserted segments was put inside hot air broiler at 65°C for 30 minutes.
 2. Deparaffinization was finished by inundating the slides in xylene for 5 minutes then in new xylene for 5 minutes.
 3. Rehydration of tissue area achieved through drenching of slides in successive weakening's of ethanol as the accompanying request:
 - I. Absolute ethanol for 5 minutes.
 - II. 95% ethanol for 5 minutes.
 - III. 90% ethanol for 5 minutes.
 - IV. 70% ethanol for 5 minutes.
 - V. 50% ethanol for 5 minutes.

VI. Distilled water for 5 minutes.

- C. Peroxidase square: Slide surrounded with Pap pen. Hydrogen peroxide was connected to cover the tissue and brooded for 20 minutes. At that point the slides were flushed with refined water, depleted and smudged delicately.
- D. Protein hindering of Non-particular official of essential neutralizer:

Before including the essential antibodies, slides were prepared for blocking advance, to square endogenous Fc receptor, brood areas for 20 min to keep any unspecific authoritative of essential immune response (FC district) with tissue segment, this with counteract false positive outcomes. At that point slides were depleted and blotched without washing.

Assessment of the Immunostaining

Assessment of IHC comes about for performed by light magnifying lens (Genex 20, America) at 40X target focal point with add up to intensity of amplification 400X. The run of the mill consequences of immunochemical recoloring found in entorhinal cortex (EC). All outcomes considered a relative level of positive cells recolored with dim darker shading out of aggregate check of positive and negative cells⁹.

Factual investigation

The factual investigations were performed with GraphPad Prism® 7.0e (USA). The qualities are exhibited as the mean ± standard deviation of the mean. The information were broke down utilizing one-route investigation of fluctuation (ANOVA) trailed by a LSD Post Hoc to recognize noteworthy contrasts between tried medication aggregate with every ordinary control, negative control and positive control gathering. The extents of recurrence of seizure and death rate were portrayed as check and rate. Factual hugeness was characterized as P≤0.05.

RESULTS

1-beginning of seizure (onset of seizure)

After pilocarpine infusion, the mean beginning of shaking in Ivabradine gather was (25.7±6.07) minutes.

Table 1: Effect of Ivabradine on the Onset of Seizure contrasted and Negative control and Valproic corrosive in Pilocarpine prompted Seizure in rats (n= 10/gathering).

	Negative	Positive	Ivabradine
Onset of seizure	9.6±2.12	27±8.11	25.7±6.07
Negative		<0.001**	<0.001**
Positive			0.996 ^{NS}

Data presented as Mean ±SD, NS: Non statistical significant difference (p>0.05); *Statistical significant difference (p≤0.05), ** highly statistical significant difference (p≤0.001).



According to the onset of seizure in present study, Ivabradine has highly significant difference ($p \leq 0.001$) when compared with negative control but with none significant difference ($p = 0.996$) and nearly comparable with Valproic acid.

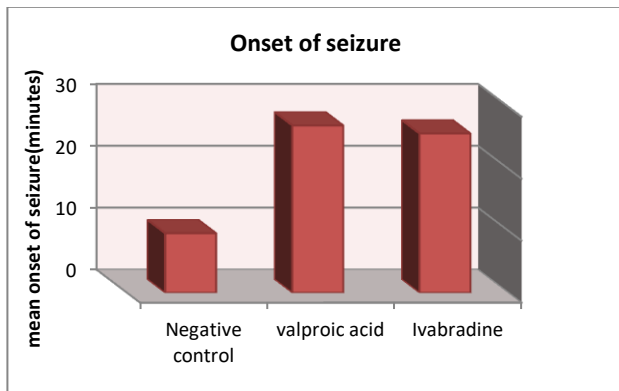


Figure 1: Effect of Ivabradine on the Onset of Seizure compared with Negative control and Valproic acid in Pilocarpine induced Seizure in rats ($n = 10/\text{group}$).

2-Duration of seizure

After pilocarpine injection, the mean duration of convulsion of Ivabradine group was (7.3 ± 1.64) seconds.

Table 2: Effect of Ivabradine on the duration of Seizure compared with Negative control and Valproic acid in Pilocarpine induced Seizure in rats ($n = 10/\text{group}$).

	Negative	Positive	Ivabradine
Duration of seizure	26.5±3.5	9.3±2.21	7.3±1.64
Negative		<0.001**	<0.001**
Positive			0.370 ^{NS}

Data presented as Mean±SD, NS: None statistical significant difference ($p > 0.05$).

*: Statistical significant difference ($p \leq 0.05$), **: Highly statistical significant difference ($p \leq 0.001$).

According to the duration of seizure in present study, Ivabradine has highly significant difference ($p \leq 0.001$) when compared with negative control but with non-significant difference ($p > 0.05$) when compared with Valproic acid.

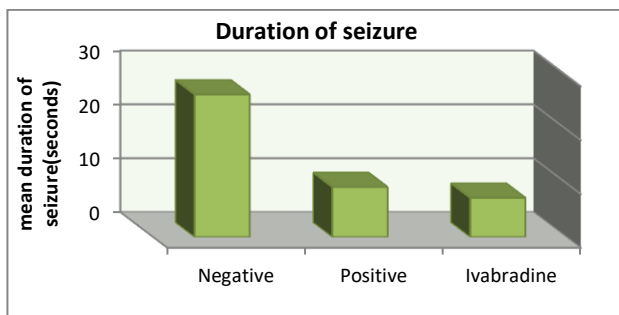


Figure 2: Effect of Ivabradine on the duration of Seizure compared with Negative control and Valproic acid in Pilocarpine induced Seizure in rats ($n = 10/\text{group}$).

3-Neu N

After pilocarpine injection, the mean of Neu N of Ivabradine group after convulsion was (75.6 ± 5.46 %).

Table 3: Effect of Ivabradine on neuron cell compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats ($n = 10/\text{group}$).

	Normal	Negative	Positive	Ivabradine
Neu N	100±0	62.1±8.54	83.5±6.64	75.6±5.46
Normal		<0.001**	<0.001**	<0.001**
Negative			<0.001**	<0.001**
Positive				0.0716 ^{NS}

Data presented as Mean±SD, NS: None statistical significant difference ($p > 0.05$).

*: Statistical significant difference ($p \leq 0.05$), **: Highly statistical significant difference ($p \leq 0.001$).

According to the Neuron antigen in present study, Ivabradine has highly significant difference ($p \leq 0.001$) when compared with normal control and negative control whereas non-significant difference ($p > 0.05$) when compared with Valproic acid.

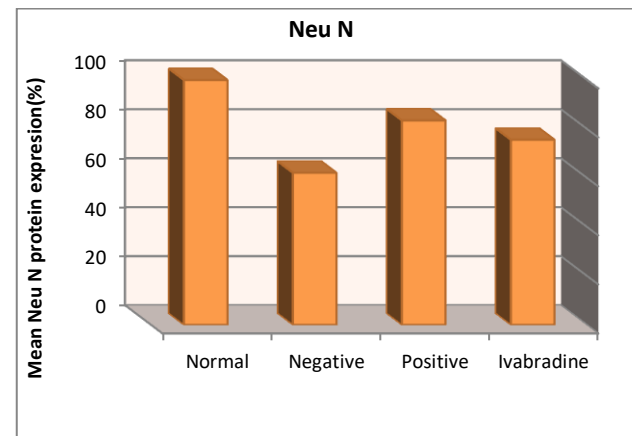


Figure 3: Effect of Ivabradine on the neuron cell compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats ($n = 10/\text{group}$).

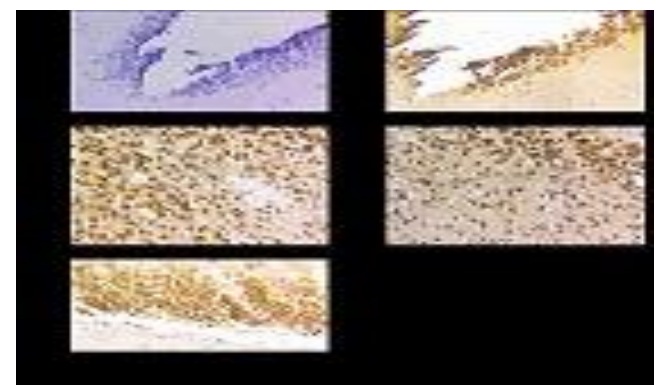


Figure 4: Showing Neu N staining using Rabbit polyclonal anti- Neu N diluted as 10pg/ml visualized by peroxidase conjugate enzyme. (A). IHC quality control (staining

without adding primary antibody) showing negative results. (B). Normal rat (without treatment or induction) showing staining of all neuronal cells with intense dark brown color. (C). Negative control (induction only), (D). Positive control (treated with Valproic acid), (E). Ivabradine treated.

4-NMDA receptors

After pilocarpine injection, the mean of NMDA of Ivabradine group after convulsion was (8.5±3.6%).

Table 4: Effect of Ivabradine on NMDA compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).

	Normal	Negative	Positive	Ivabradine
NMDAR	3.9±1.2	33.4±9.5	9.8±4.69	8.5±3.6
Normal		<0.001**	0.132 ^{NS}	0.391 ^{NS}
Negative			<0.001**	<0.001**
Positive				0.994 ^{NS}

Data presented as Mean±SD, NS: None statistical significant difference (p>0.05).

*: Statistical significant difference (p≤0.05), **: Highly statistical significant difference (p≤0.001).

According to NMDA receptor in present study, Ivabradine has non-significant difference (p≤0.001) when compared with normal control and Valproic acid whereas highly significant difference (p>0.05) when compared with negative control.

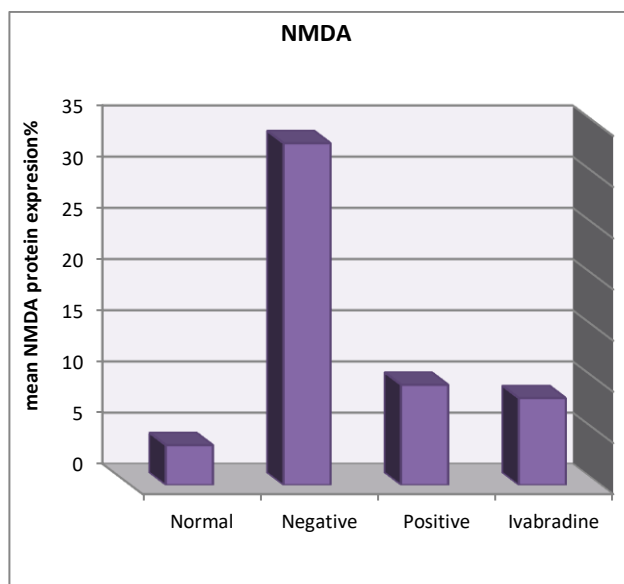


Figure 5: Effect of Ivabradine on the NMDA receptor compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).

5-Nav 1.6

After pilocarpine injection, the mean of Nav1.6 of Ivabradine group after convulsion was (8±3.02%).

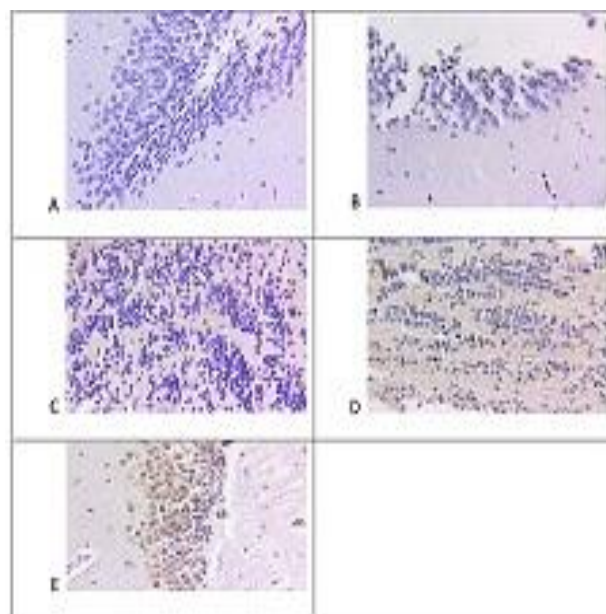


Figure 6: Showing NMDA receptor staining using Rabbit polyclonal anti- NMDA antibody diluted as 10pg/ml visualized by peroxidase conjugate enzyme. (A). IHC quality control (staining without adding primary antibody) showing negative results. (B). Normal rat (without treatment or induction) showing staining of all neuronal cells with intense dark brown color. (C). Negative control (induction only), (D). Positive control (treated with Valproic acid), (E). Ivabradine treated.

Table 5: Effect of Ivabradine on the Nav1.6 compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).

	Normal	Negative	Positive	Ivabradine
Nav1.6	2.1±1.29	28.5±7.25	10.4±4.12	8±3.02
Normal		<0.001**	0.003*	0.088 ^{NS}
Negative			<0.001**	<0.001**
Positive				0.893 ^{NS}

Data presented as Mean±SD, NS: None statistical significant difference (p>0.05).

*: Statistical significant difference (p≤0.05), **: Highly statistical significant difference (p≤0.001).

According to the Nav1.6 in present study, Ivabradine has non-significant difference (p≤0.001) when compared with normal control and Valproic acid whereas highly significant difference (p>0.05) when compared with negative control.

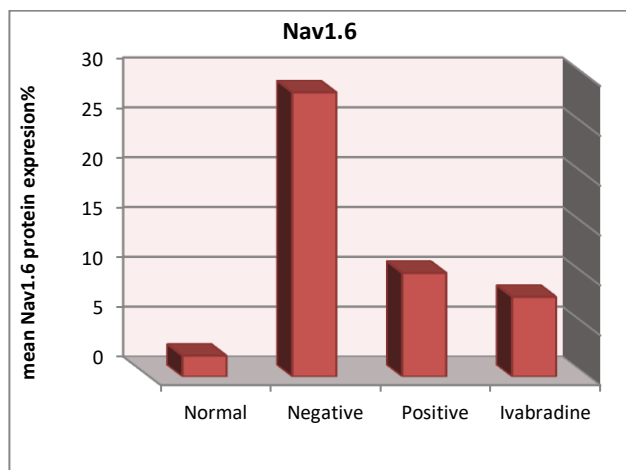


Figure 7: Effect of Ivabradine on the Nav1.6 compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).

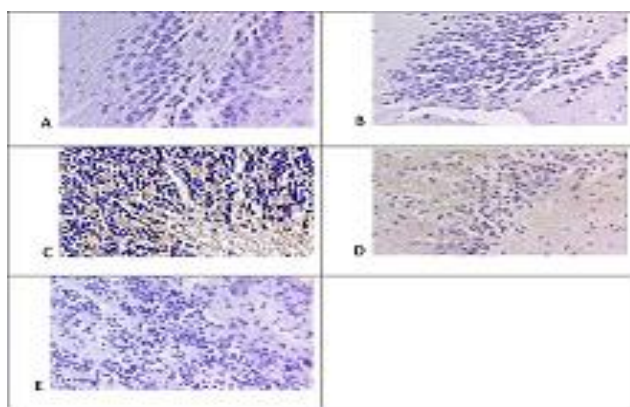


Figure 8: Showing Nav 1.6 receptor staining using Rabbit polyclonal anti- Nav 1.6 antibody diluted as 10pg/ml visualized by peroxidase conjugate enzyme. (A). IHC quality control (staining without adding primary antibody) showing negative results. (B). Normal rat (without treatment or induction) showing staining of all neuronal cells with intense dark brown color. (C). Negative control (induction only), (D). Positive control (treated with valproic acid), (E). Ivabradine treated

DISCUSSION

Epilepsy is a standout amongst the most widely recognized neurological issues everywhere throughout the world, being related with paroxysmal release of cerebral neurons and is portrayed by a few indications including adjustments of practices and awareness managed change in mind work¹⁰. The pilocarpine gives a valuable creature model to considering systems and helpful ways to deal with epilepsy. In this model, exorbitant and maintained incitement of cholinergic receptors can prompt seizure-related mind harm in rodents¹¹.

Impact of Ivabradine

A few sorts of epilepsy have a hereditary segment mostly connected to transformations in qualities encoding voltage-gated (Na⁺, Ca²⁺, and K⁺) or ligand-gated (GABAA

and cholinergic nicotinic receptor) channels¹². Na diverts are novel in that they are dually enacted by voltage hyperpolarization and intracellular cAMP. These diverts have built up part in tweaking neuronal edginess¹³. The present examination surveyed the anticonvulsant impact of Ivabradine, as sodium channels, NMDA receptor blocker, in Pilocarpine-prompted seizure in rats. The consequences of the present work uncovered that, seizure intensity and length are diminished by Ivabradine while expanded the idleness to seizures. While, Valproic corrosive diminished seizure strength more than Ivabradine, the impact of the two medications on dormancy and span of seizure were inconsequential unique. These outcomes concur with Luszczi *et al*¹⁴ who found that Ivabradine expanded the edge for maximal electroshock-instigated tonic seizures in mice. They gathered that Ivabradine, because of its Na channel blocking properties, can bolster the ionic homeostasis in the mind, and along these lines, the medication raised the limit for electro convulsions in mice. In help of these discovering, Bender *et al*¹⁵ hypothesized that the resected hippocampi from patients with transient projection epilepsy, demonstrated improvement in the levels of Na channel articulation with dendritic limitation in granule cells of the dentate gyrus. Moreover, another research has distinguished a change in the HCN2 quality with growth of Na current in patients with hereditary epilepsy and had febrile seizures Dibbens *et al*¹⁶. Be that as it may, Poolos *et al*¹⁷, who announced the expansion in Na current may be credited to improvement of neuronal hyperpolarization due actuation of GABA receptor. The subsequent film hyperpolarization is a very much recorded jolt for opening HCN channels and Na current event. Surely, in different kinds of neurons the voltage-enacted Na⁺/K⁺ "Ih" current has been recognized as depolarizing present and actuated by film hyperpolarization encouraged by cAMP¹⁸. Postea and Biel¹⁹ proposed that the Hyperpolarization-enacted cyclic nucleotide-gated (HCN) channels are included not just in synaptic transmission and neuronal volatility under physiological conditions, yet additionally in seizure action. To decide the impact of Ivabradine (a HCN channel inhibitor) on the anticonvulsant power of four novel antiepileptic drugs (AEDs: lacosamide, lamotrigine, pregabalin and topiramate) in the mouse maximal electroshock-prompted seizure (MES) show. Grown-up male pale skinned person Swiss mice were tested with maximal electroconvulsions. Add up to mind centralizations of AEDs were estimated with high-weight fluid chromatography. Ivabradine (10 mg/kg, I.P) essentially decreased the anticonvulsant power of lamotrigine. Conversely, Ivabradine (10 mg/kg, I.P) did not altogether influence the anticonvulsant power of lacosamide, pregabalin or topiramate in the mouse MES show. Furthermore, Ivabradine had no effect on add up to cerebrum centralizations of all the examined AEDs in mice. An extraordinary alert is exhorted when consolidating Ivabradine with lamotrigine in epileptic patients because of the conceivable pharmacodynamics

diminishment of the anticonvulsant activity of the later medication. The mixes of Ivabradine with lacosamide, pregabalin and topiramate appear to be pharmacodynamic and nonpartisan from a preclinical perspective.

CONCLUSION

Ivabradine has a legitimate preventive impact against seizures prompted by Pilocarpine in rodent which were tantamount to that of valproate corrosive.

REFERENCES

- Amudhan S, Gururaj G, Satishchandra P. Epilepsy in India I: Epidemiology and public health. *Ann Indian Acad Neurol.* Jul-Sep, 18(3), 2015, 263-77.
- Meador KJ, Loring DW. Developmental effects of antiepileptic drugs and the need for improved regulations. *Neurology.* Oct, 2015, 30.
- Yancy, CW; Jessup, M; Bozkurt, B; Butler, J; et al. (20 May 2016). "2016 ACC/AHA/HFSA Focused Update on New Pharmacological Therapy for Heart Failure: An Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America". *Circulation.* 134, e282–e293.
- Sulfi S, Timmis AD (2006). "Ivabradine – the first selective sinus node If channel inhibitor in the treatment of stable angina". *Int. J. Clin. Pract.* 60 (2), 222–8.
- Glien M, Brandt C, Potschka H, Voigt H, Ebert U, Löscher W. Repeated low-dose treatment of rats with pilocarpine: low mortality but high proportion of rats developing epilepsy. *Epilepsy Res.* 2001, 46(2), 111-119.
- Minicucci F, Muscas G, Perucca E, Capovilla G, and Vigeveno F, Tinuper P. Treatment of Status Epilepticus in Adults: Guidelines of the Italian League against Epilepsy. *Epilepsia.* 47(s5), 2006, 9-15.
- Schauwecker PE.: Strain differences in seizure-induced cell death following pilocarpine-induced status epilepticus. *Neurobiol. Dis.*; 45(1), 2012, 297–304.
- Kalyuzhny AE. *Immunohistochemistry: Essential Elements and Beyond.* Springer International Publishing; 2016.
- Shao H, Yang Y, Mi Z, et al. Anticonvulsant effect of Rhyncophylline involved in the inhibition of persistent sodium current and NMDA receptor current in the pilocarpine rat model of temporal lobe epilepsy. *Neuroscience.* 337(September), 2016, 355-369.
- Freitas RM., Sousa FC F., Vasconcelos SMM., Viana GSB. and Fonteles MMF. Pilocarpine-induced status epilepticus in rats: lipid peroxidation level, nitrite formation, GABAergic and glutamatergic receptor alterations in the hippocampus, striatum and frontal cortex. *Pharmacology, Biochemistry and Behavior,* 78, 2004, 327–332.
- Khongsombat O., Tantisira B. and Tantisira MH. Effects of N (2-propylpentanoyl) urea on hippocampal amino acid neurotransmitters in spontaneous recurrent seizure rats. *Asian Biomedicine;* 4, 2010, 271-279.
- Thomas RH, Berkovic SF The hidden genetics of epilepsy-a clinically important new paradigm. *Nat Rev Neurol* 10, 2014, 283–292.
- Vilaine JP The discovery of the selective If current inhibitor ivabradine. A new therapeutic approach to ischemic heart disease. *Pharmacol Res* 53, 2006, 424–434.
- Luszczki JJ, Prystupa A, Andres-Mach M, Marzęda E, Florek-Luszczki M (Ivabradine) a hyperpolarization activated cyclic nucleotide-gated channel blocker) elevates the threshold for maximal electroshock-induced tonic seizures in mice. *Pharmacol Rep,* (5) 65, 2013, 14-1407.
- Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. *J Neurosci* 23, 2003, 6826–6836.
- Dibbens LM, Reid CA, Hodgson B, Thomas EA, Phillips AM, Gazina E, Cromer BA, Clarke, AL, Baram TZ, Scheffer IE Augmented currents of an HCN2 variant in patients with febrile seizure syndromes. *Ann Neurol* 67, 2010, 542–546.
- Poolos NP, Migliore M, Johnston D Pharmacological upregulation of Ih channels reduces the excitability of pyramidal neuron dendrites. *Nat Neuro sci* 5, 2002, 767–774.
- Accili EA, Proenza C, Baruscotti M, DiFrancesco D From funny current to HCN channels: 20 years of excitement. *News Physiol Sci* 17, 2002, 32–37.
- Postea O, Biel M Exploring HCN channels as novel drug targets. *Nat Rev Drug Discov* 10, 2011, 903–914.

Source of Support: Nil, Conflict of Interest: None.

