

ROLE OF MULTIPLE ION CHANNEL BLOCKER –AMIODARONE IN MODEL OF CONVULSION, LOCOMOTOR ACTIVITY AND COGNITION IN ALBINO RATS

LOKRAJ SUBEDEE*, RN SURESHA, MD. SIBGATULLAH, SIDDAMMA A., BRAHADEESH M

Department of Pharmacology, JSS Medical college, Mysore, Department of pharmacology, Jss Medical college, Mysore, Karnataka 570015, India. Email: Subedeelok@gmail.com

Received: 19 Dec 2013, Revised and Accepted: 10 Feb 2014

ABSTRACT

Objective: To study the effect of amiodarone in experimentally induced convulsion and its effect on cognition and loco motor activity in animal models.

Methods: 24 Male swiss albino rats were taken and were maintained at 12 hr dark light cycle. The animals were acclimatized to laboratory environment 24 hr prior to the experiment. Test drug Amiodarone at doses of 100mg/kg and 150mg/kg was given i.p. Diazepam was used as Standard drug at a dose of 0.6mg/kg i.p. for both locomotor and anticonvulsant activity. Drugs were administered one hour prior to experiment. Convulsions were induced by PTZ(70mg/Kg body weight). Latency of convulsion and severity of seizure were noted. For locomotor activity rota rod test was performed and latency to fall from the rotating rod in the rota rod apparatus was noted. For cognition activity, Morris water maze test was performed where the escape latency time was noted.

Results: In PTZ Induced seizure model amiodarone showed significant increase in latency to convulsions at 135% and 168% with dose of 100mg/kg and 150mg/kg body weight respectively with compared to control and no death were reported. In case of Morris water maze model for cognition there was significant decrease in the latency to reach the platform indicating reduction in cognition level. Whereas in rotarod model for locomotor activity there was no significant change in latency to fall from rotating rod indicating no significant effect on skeletal muscle relaxant property.

Conclusion: In this study it is shown that Amiodarone have significant anticonvulsant activity without having skeletal muscle relaxant property though have side effect as cognition reducer.

Keywords: Amiodarone, Anticonvulsant, Phentyleteterazole (PTZ), Rotarod, Morris water maze, cognition, locomotor activity

INTRODUCTION

A seizure (from Latin "sacire", to take possession of) is a paroxysmal event due to abnormal, excessive, hyper synchronous discharges from an aggregate of central nervous system neurons. Depending on the distribution of discharges, this abnormal CNS activity can have various manifestations, ranging from dramatic convulsions to only EEG changes. Epilepsy describes a condition in which a person has recurrent seizures. This definition implies that a person with a single seizure does not necessarily have epilepsy. It refers to a clinical phenomenon rather than a single disease entity, since there are many forms and causes of epilepsy [1].

The causes of epilepsy are many, ranging from idiopathic to infection to neoplasm and to head injury. In some, heredity has proved to be a predominant factor [2]. Epilepsy is a major public health issue in many nations and the frequency of epilepsy and seizures increases in later life. Despite the massive scale of the problem and much research, epilepsy remains poorly understood [1].

Presently available therapy is symptomatic, i.e. the drugs inhibit seizures, but whether any of these prevent the development of epilepsy (epileptogenesis) or cure is uncertain. Insights that promise to provide molecular targets for both symptomatic and preventive therapies are being researched [3]. Although more than 20 approved drugs and several non-pharmacological options are available, up to 30% of patients are still refractory to treatment. Due to the heterogeneity of the disease and our limited understanding of it, discovery and development of anti-epileptic drugs has been especially difficult [1]. Calcium ion participates in the regulation of neural transmission and the presynaptic release of neurotransmitters. It is also involved in epileptic events, cardiac arrhythmias and abnormal conduction of stimuli. Amiodarone hydrochloride is a potent anti-arrhythmic agent, known as a multiple ion-channel blocker in the heart. Although it has been detected in the rat brain, there are no data related to its central nervous system (CNS) effects. study reported that it increased the concentrations of γ -aminobutyric acid (GABA) and glycine and decreased those of

aspartate and glutamate in rat medulla oblongata [4]. Inhibition or excitation of a neuron depends on concentrations of intracellular Ca^{2+} and Na^+ and extracellular K^+ , and also on balance between GABAergic and adenosinergic inhibitory transmissions and glutamatergic excitatory transmission. In epilepsy, cognition and locomotor activity ion channels and neurotransmitters have important roles. Since amiodarone has multiple ion-channel blocker properties and increases the inhibitory neurotransmitters, we investigate its effect on convulsion, locomotor activity and cognition.

METHODS AND MATERIALS

After approval from the Institutional Ethics Committee (JSSMC/PR/IAEC/18/26), Swiss albino rats (24) weighing around 150-200 g of either sex were randomly selected from central animal facility, J S S Medical College, Mysore. Animals were provided free access to tap water and commercial food, and were maintained under standard laboratory conditions with natural light and dark cycle, under room temperature.

They were acclimatized for 24 hours before the start of the experimentation. Animals are administered both the standard and the test drugs intra peritoneal.

Experiments were performed in accordance with the recommendations from the Declaration of Hel-sinki (National Institutes of Health, 1986) and the internationally accepted principles in the care and use of experimental animals.

Group 1- Control - Distill water Group 2-Standard- Diazepam (0.5mg/Kg body weight) Group 3- Test 1 - Amiodarone (100mg/kg body weight) Group 4- Test 2- Amiodarone (150mg/kg body weight)

Drugs

Amiodarone hydrochloride (wockhardt, India), PTZ (Sigma, USA), diazepam (Cipla, India), used in the study were dissolved in distilled water, in a volume of 0.1 ml/10 g, intraperitoneally (i.p.).

PTZ-induced generalized convulsion models

Pentylenetetrazol is a tetrazol derivative with consistent convulsive effect in a large number of animal like mice, rats, cats, primates etc. It is believed to act by antagonizing the inhibitory GABAergic neurotransmission. PTZ test is used for screening of drugs effective in petit mal epilepsy or absence seizures. Animals were divided in to three groups 6 rats each group. Control group received the distilled water, standard group received the Diazepam (0.6mg/Kg) and amiodarone (100mg/kg) i.p. the dose was taken from previous studies. All the animals were individually placed in plastic cages for observation during 30 min for latency (the time period until the onset of the loss of righting reflex followed by a long tonus) and time to death (the time period until the death)[5] and these periods were measured as second(s). The convulsion rates and the mortality were also evaluated for each group.

Morris water maze cognition model.

It is a very popular test for spatial learning and memory and cognition. Spatial learning and memory was tested in a water maze following the method of Pappas *et al*[6] 1996. The water maze consists of a pool, with a platform. The entire animals were trained for swimming for 5 days prior to the experiment. Each rat was placed in the water facing the wall at the start location and was allowed 90 seconds to find the hidden platform. The animal was allowed a 30-second rest on the platform. If the rat was unable to locate the hidden platform, it was lifted out and placed on the platform for 30 seconds. Here the test group had given amiodarone of different dose 100mg/kg body weight and 150 mg/kg bodyweight for 3 days prior to the test. On the sixth day test was performed after half an hour of administration of drug and latency of time to reach platform was noted for each group of rats.

Rota rod test for locomotor activity

Rotarod test is used to assess the effect of drugs on the motor coordination or fatigue resistance on mice and rats using one unit in a safe and humane way. The animals are placed on textured drums to avoid slipping. When an animal drops onto the individual sensing platforms below the plate trips and corresponding magnetic switch is activated, thereby recording the animal's endurance (staying power) time in seconds. The test results are recorded digitally and displayed on the front panel. All the animals were trained for the Rota rod test prior to the experiment for two days (5 min session each). Test was performed after half an hour of administration of drugs and Latency of time falling from rotating rod were recorded.

RESULTS

Effects of amiodarone on the latency of convulsion

In Ptz induced model of convulsion, the amiodarone at dose of 100mg/kg body weight and 150mg/kg body weight significantly increased the latency of convulsion with respect to increase dose effect and no death were reported. while with standard drugs there was no convulsion. The latency of convulsion was increased 135 % in case of 100mg amiodarone group and 168 % in case of 150 mg amiodarone group with compared to control where 4/6 animal died. The result was significant statistically significant ($p < 0.05$) as shown in table 1.0. while in diazepam group convulsion was 100% controlled.

Table 1.1: Table showing latency of convulsion in different groups of rats

Groups	No of Animals	Mean \pm SD	Death rate
Control	6	3.50 \pm 1.04	4/6
Test 1	6	10.33 \pm 1.36	0/6
Test 2	6	13.33 \pm 0.81	0/6
Standard	6	No convulsion	0/6

Effects of amiodarone on locomotor activity

In the rotarod model of locomotor activity there was no significant decrease in latency time to fall from rotating rod showing 5% decrease with dose at 100 mg/kg body weight and 8% decrease with

dose at 150 mg/kg body weight compared to control. This indicates amiodarone does not have skeletal muscle relaxant property. Whereas with standard drug showed 400% decrease in latency to fall from rotating rod in comparison to control (Table 1.2). Results were significant at $p < 0.05$.

Table 1.2: Table showing latency of time falling from rota rod in different groups

Groups	No. of animals	Mean \pm SD	ANOVA
Control	6	62.0 \pm 4.09	
Standard	6	37.5 \pm 3.44	F =61.67
Test 1	6	58.5 \pm 3.61	P = 0.001
Test 2	6	59.5 \pm 2.88	

Effect of amiodarone in cognition

In Morris water model of cognition there is significant increase in latency time showing 26% with dose at 100 mg/kg body weight and 66.3 % with dose at 150 mg/kg body weight compared to control. This represents amiodarone has dose dependent effect on cognition (Table 1.3). all the results were significant statistically at $P < 0.005$.

Table 1.3: Table showing Escape latency time of different groups of animals

Groups	No. of animals	Mean \pm SD	ANOVA
Control	6	14.33 \pm 1.50	
Test 1	6	18.16 \pm 1.94	F=43.74
Test 2	6	23.83 \pm 1.83	P=0.001

DISCUSSION

PTZ-induced model, the most popular and widely used animal seizure model, represents a valid model for human generalized myoclonic and also absence seizures, and this test has been used primarily to evaluate anticonvulsant drugs [7]. Although convulsive activity of PTZ is not fully understood, it has been reported that PTZ has induced seizures by inhibiting GABA pathway in CNS [8] acting as an antagonist at GABA-A receptor complex increasing the central noradrenergic activity, and increasing the intracellular calcium and extracellular potassium ion concentrations. As shown in the Fig.1, in the PTZ-induced model, amiodarone administrations prolonged the latency to seizure, and decreased the convulsion rates with no mortality. here our test reveals that the test drug amiodarone have anticonvulsant activity decreasing the severity of seizure, increasing latency of inducing seizures as well as no death of animals. (Table 1.1 and 1.2). Turovaya *et al.* (2005)[4] reported that amiodarone had increased the concentrations of inhibitory GABA and glycine and had decreased those of excitatory aspartate and glutamate in rat medulla oblongata. In our study, the anticonvulsant effect of amiodarone in PTZ-induced seizures may be partially due to blockage of ion channels (Na^+ , K^+ and Ca^{2+}) and/or its involvement in noradrenergic pathways and/or in GABAergic pathway and/or its anti-oxidant and anti-inflammatory effects (COX-2 inhibition.)

Memory -As shown in graph (2.1) the effect have the effect on cognition showing reduction is latency time to reach the platform in morris water maze test, this is due the effect on GABA receptors. GABA_A receptors are known to down regulate memory consolidation processes: picrotoxin and bicuculline enhance memory, and benzodiazepines and muscimol depress it. We speculate that amiodarone may prevent the adenosine receptor blockage, increase GABA-A receptor blockage and decrease of NMDA receptor neurotransmission.

Locomotor activity-Although amiodarone is a calcium channel blocker, it does not effect on peripheral skeletal muscles (Graph 3.1) so we came to conclusion that it does not have skeletal muscle relaxant property, same other calcium channel blocker optical isomers of verapamil vary considerably in their potency as Ca^{2+} channel blockers, they were equally effective inhibitors of insulin-

stimulated glucose transport rate. Nifedipine (10-200 microM), a more potent blocker of skeletal muscle Ca²⁺ channels than verapamil, was less effective as an inhibitor of insulin-stimulated glucose transport. Furthermore, nifedipine (10 microM) did not inhibit hypoxia-stimulated glucose transport. Diltiazem (200 microM), another Ca²⁺ channel blocker, did not reduce insulin-stimulated glucose transport[9].

CONCLUSION

In this study, we have noted that amiodarone had a significant anticonvulsant property without any effect on locomotor function. Though significant reduction on cognition levels were noted. These results indicate that amiodarone has CNS effects, especially central depressant effects and no skeletal relaxant property however, further studies are necessary to investigate more effects and adverse effects.

ACKNOWLEDGEMENT

I acknowledge all the staffs and my postgraduate colleagues of Pharmacology department of JSS Medical College, Mysore for guiding me in this project.

REFERENCES

1. Lowenstein DH: Seizures and epilepsy. In, Fauci, Brainwald, Kasper, Hauser, Longo, Jameson. Harrisons Principles of Internal Medicine, 17th edition. USA, McGraw Hill; 2008: 2498.
2. Porter RJ, Meldrum BS. "Antiseizures Drugs". In, Katzung BG, Masters SB, Trevor AJ. Basic and Clinical Pharmacology, 11th edition. New Delhi, Tata-McGraw Hill; 2009: 399.
3. McNamara JO. Pharmacotherapy of epilepsies. In, Brunton LL, Lazo JS, Parker KL. Goodman & Gillman's The pharmacological basis of Therapeutics, 11th edition. U S A, McGraw Hill; 2006: 501.
4. Turovaya, A.Y., Galenko-Yaroshevskii, P.A., Kade, A.K., Uvarov, A.E., Kiguradze, M.I., Khvitiya, N.G., Tatu-lashvili. Effects of verapamil and amiodarone on sympathoadrenal system and balance of excitatory and inhibitory amino acids in rat medulla oblongata. *Bull. Exp. Biol. Med.* 2005, 139(6):665-667.
5. Dhanabal, S.P., Paramakrishnan, N., Manimaran, S., Suresh, B. Anticonvulsant potential of essential oil of ar-temisia abrotanum. *Curr. Trends Biotechnol. Pharm.* 2007,1(1):112-116.
6. Pappas BA, De La Torre JC, Davidson CM, Keyes MT, Fortin T. Chronic reduction of cerebral blood flow in the adult rat: Late emerging CA1 cell loss and memory dysfunction. *Brain Res* 1996;708:50-8
7. Loscher, W., Schmidt, D., Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Res.* 1988, 2(3):145-181.
8. Corda, M.G., Giorgi, O., Longoni, B., Orlandi, M., Biggio, G. Decrease in the function of the γ -aminobutyric acid-coupled chloride channel produced by the repeated administration of pentylentetrazol to rats. *J. Neurochem.* 1990.
9. Cartee GD, Briggs-Tung C, Holloszy JO. Biodynamics Laboratory, University of Wisconsin-Madison 53706, : Am J Physiol. 1992 Jul;263(1 Pt 2):R70-5.