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Research Article

Comparative Effect of Two Anticonvulsants (Magnesium Sulphate and Diazepam) on 4-Aminopyridine - Induced Seizures in CD1 Mice

Ofutet, E.O[1]*., Mfem, C.C. [1], Okpo-ene, I.A.[1], Agu C.E. [2]
[1]Department of Chemical pathology, University of Calabar Teaching Hospital, Calabar, Cross River state, Nigeria
[2]Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, P.M.B. 1115 Calabar, Cross River State, Nigeria

***Corresponding Author:**
Ofutet EO

Email: emazoeagape@gmail.com

Abstract: Four-aminopyridine (4-AP) is a potassium channel blocker often used in the management of some neurological disorders, and is often accompanied with seizures as one of its side effect. This study comparatively assessed the effects of two anticonvulsants ($MgSO_4$ and Diazepam) on seizures induced by 4-AP in CD1 mice, with a view to possibly recommend the combined administration of 4-AP with any of these anticonvulsants to ameliorate convulsions (one of 4-AP's side effects). Twenty one (21) CD1 mice weighing 20 – 25g were used for this study. They were assigned into 3 groups (n = 7) as follows; control seizure (13.3mg kg^{-1} of 4-AP), seizure + diazepam (2mg kg^{-1}), and seizure + $MgSO_4$ (4.5mg kg^{-1}) groups. Induction of seizures was done 30 minutes after the intramuscular and subcutaneous administration of the anticonvulsants ($MgSO_4$ and diazepam, respectively), after which parameters such as; the onset of trembling, wild running, jerking, tonic clonic seizures and time of death, were evaluated using standard methods. The results obtained showed that $MgSO_4$ and Diazepam delayed the onset of seizures by significantly ($p<0.001$) increasing the onset of trembling, wild running, jerking, tonic clonic seizures and significantly ($p<0.001$) delayed time of death compared with control. $MgSO_4$ significantly ($p<0.001$) increased the onset of trembling, wild running, jerking, tonic clonic seizures and delayed the time of death compared to diazepam. Both anticonvulsants delayed the onset of the events that bring about seizures induced by 4-AP, but none could completely reverse seizures; therefore the combined administration of 4-AP and either of these anticonvulsants may not offer any therapeutic benefit following seizures induced by 4-AP**.**

Keywords: Convulsion, diazepam, magnesium sulphate, seizures, 4-aminopyridine.

INTRODUCTION

Four-aminopyridine (4-AP) is a potassium channel blocker often employed in the management of cerebellar ataxia, Lambert–Eaton syndrome, myasthenia gravis, tetrodotoxin-induced paralysis, anesthesia-induced neuromuscular blockade, botulism [1, 2], and multiple sclerosis [3]. In demyelinated neurons, 4-AP has been shown to improve both axonal conduction and synaptic transmission and, therefore, is of notional therapy for spinal cord injury [4-6]. It facilitates calcium conductance by blocking potassium channels on the cytoplasmic side of the cellular membrane, causing depolarization and opening of voltage-gated calcium channels [7, 8]. 4-AP readily penetrates the blood-brain barrier and is believed to induce seizure activity by enhancing spontaneous and evoked neurotransmitter release. In mice, parenterally administered 4-AP induces clonic-tonic convulsions, wild running, tonic hind limb extension and lethality [9].

Diazepam is a benzodiazepine that is widely used in the management of an array of disorders such as, anxiety, convulsion, insomnia, panic attack, symptoms of acute alcohol withdrawal [10]. It is also used in the treatment of different states of agitation, neurodegenerative disorders like vertigo, tetanus [11, 12], palliative treatment of stiff person syndrome, adjunct treatment of spastic muscular paresis caused by spinal cord or cerebral conditions such as multiple sclerosis, stroke and spinal cord injuries. Also, it can be very preventive in combating with conditions like oxygen toxicity during hyperbaric oxygen therapy [13]. Diazepam, like other benzodiazepines is a positive allosteric modulator of GABA type A receptors. These receptors are ligand-gated selective chloride ion channels that are activated by Gamma-amino butyric acid (GABA), one of the main inhibitory neurotransmitter in the central nervous system. Binding of diazepam to this receptor complex promotes binding of GABA, which in turn increases the total conduction of chloride ions across the neuronal cell membrane.

Magnesium sulphate ($MgSO_4$) is a naturally occurring mineral that is important in many systems of the body especially the muscles and the nerves. It is an important co - factor for enzymatic reactions and plays an important role in neurochemical transmission and muscular excitability. Magnesium sulphate injection is largely used clinically for treatment of lots of ailments including; constipation, hypomagnesemia, pre-eclampsia, eclampsia, pregnancy toxaemia as well as convulsions [14, 15] . As a nutritional adjunct in hyper-alimentation, its precise mechanism of action is uncertain. However, it has been reported that it prevents and controls convulsion by blocking neuromuscular transmission and decreasing the amount of acetylcholine liberated at the end plate by the motor nerve impulse [16]. In view of it multifaceted clinical benefits of 4-P, it has been implicated with side effects such as abdominal pain, paraesthesias, hyperexcitability, hyper-salivation, diaphoresis and convulsions (seizures). Our study was therefore aimed at comparatively assessing the effects of two anticonvulsants ($MgSO_4$ and Diazepam) on seizures induced by 4-AP in CD1 mice, with a view to possibly recommend the combined administration of 4-AP with any of these anticonvulsants to ameliorate convulsions (one of 4-AP's side effects).

METHODOLOGY

Experimental Design

Twenty one (21) healthy albino mice of both sexes weighing between 20-25g were used for this study. The animals were purchased from the animal house of the Department of Physiology, Faculty of basic medical science, University of Calabar, Calabar. They were kept in well ventilated cages at room temperature with 12hour dark/light cycle. The animals were allowed access to feed and water *ad libitum.* The animals were kept in hygienic environment with their bedding changed every morning until the date of the experiment After seven (7) days of acclimatization [17, 18], there were assigned into 3 groups as follows; Ccontrol, Diazepam treated and $MgSO_4$ treated groups (n = 7). The experimental animals were handled following the laid down principles of Helsinki, 1964.

Drug Administration

The test drugs for this study were administered intraperitonnally, subcutaneously and intramuscularly for 4-AP (13.3mg/kg), diazepam (2.0mg/kg) and $MgSO_4$ (4.5mg/kg) respectively.

Induction of Seizure

Thirty (30) minutes after animals were treated with diazepam and Magnesium sulphate, seizure was induced by intraperitoneal administration of 4-AP at a dose of 13.3mg/kg body weight as described by Yamagushi & Rogawski [19]. Parameters of seizures such as the onset of trembling, wild running, jerking, tonic clonic seizures and time of death, were evaluated using standard accordingly with the aid of a video camera and an electronic stop watch.

Statistical Analysis

Data obtained were expressed as mean ± SEM. The statistical analyses were done using the analysis of variance (ANOVA) and the post/hoc Newman Keul's test. A difference between means was considered significant at $p < 0.05$.

RESULTS

Onset of Trembling

The mean values of the onset of trembling for the control, seizure treated with magnesium sulphate (MS) and seizure treated with diazepam (DZP) are 81.50 ± 2.29, 282.17 ± 17.3 and 207.83 ± 3.68 seconds, respectively. With MS treated group having a significant increase ($p<0.001$) in the onset of trembling compared with control. DZP treated group also has a significant increase ($p<0.001$) in onset of trembling compared with control, and a significant ($p<0.001$) decrease in the onset of trembling compared with MS treated group (Figure 1).

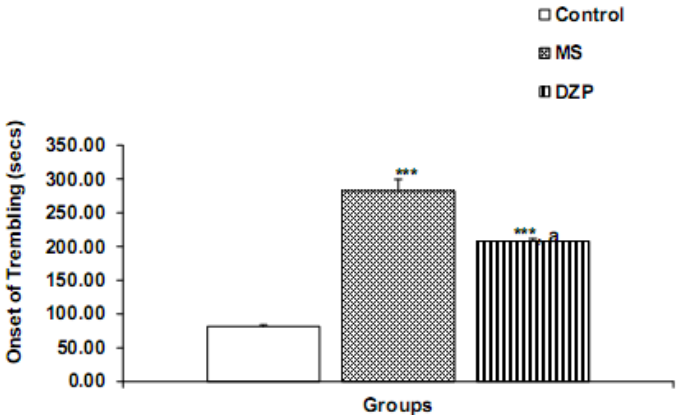

Fig-1: Comparison of onset of trembling in the different experimental groups

Values are mean ± SEM, n = 7.

***p<0.001 vs control; a = p<0.001 vs MS.

Onset of Wild Running

The mean values for the onset of wild running for the control, seizures treated with MS and seizures treated with DZP are 300.50 ± 5.49, 502.00 ± 4.74 and 425.83 ± 17.56 seconds, respectively. The onset of wild running is significantly ($p<0.001$) higher in the MS treated group compared with control. DZP treated group has a significant increase ($p<0.001$) in onset of wild running compared with control, but is significantly decreased ($p<0.001$) compared with MS treated group (Figure 2).

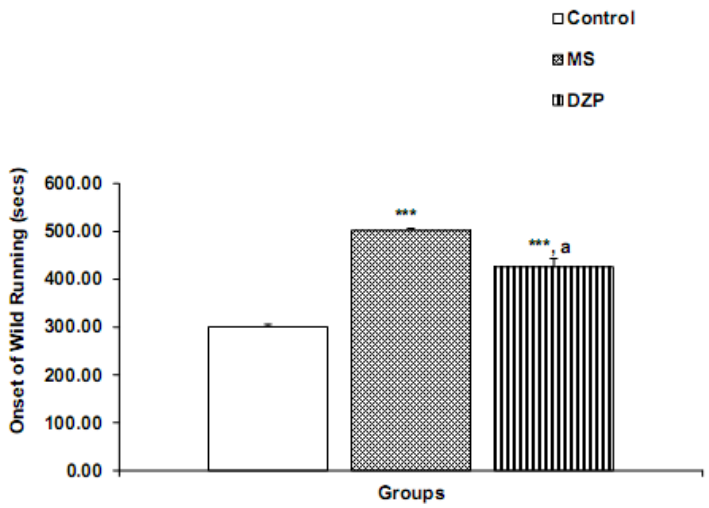

Fig-2: Comparison of onset of wild running in the different experimental groups

Values are mean ± SEM, n = 7.

***p<0.001 vs control; a = p<0.001 vs MS.

Onset of Jerking

The mean values for the onset of jerking for the control, seizures treated with MS and seizures treated with DZP are 32.17 ± 2.73, 130 ± 4.55 and 79 ± 2.86 seconds, respectively. The onset of jerking is significantly ($p<0.001$) higher in the MS treated group compared with control. DZP treated group also has a significant increase ($p<0.001$) in onset of jerking compared with control, but is significantly decreased ($p<0.001$) compared with MS treated group (Figure 3).

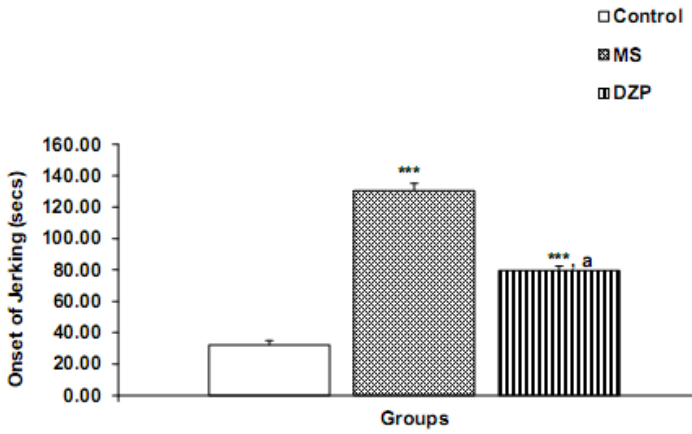

Fig-3: Comparison of onset of jerking in the different experimental groups

Values are mean ± SEM, n = 7.

***p<0.001 vs control; a = p<0.001 vs MS.

Onset of Tonic Clonic Seizure

The values for the onset of tonic clonic seizure for the control, MS treated and DZP treated groups are 32.17 ± 2.73, 130.50 ± 4.55 and 79.67 ± 2.86 seconds, respectively. MS treated group has a significant ($p<0.001$) increase in the onset of tonic clonic seizure compared with control. DZP treated group has a significant increase ($p<0.001$) in onset of tonic clonic seizure compared with control, but is significantly decreased ($p<0.001$) compared with MS treated group (Figure 4).

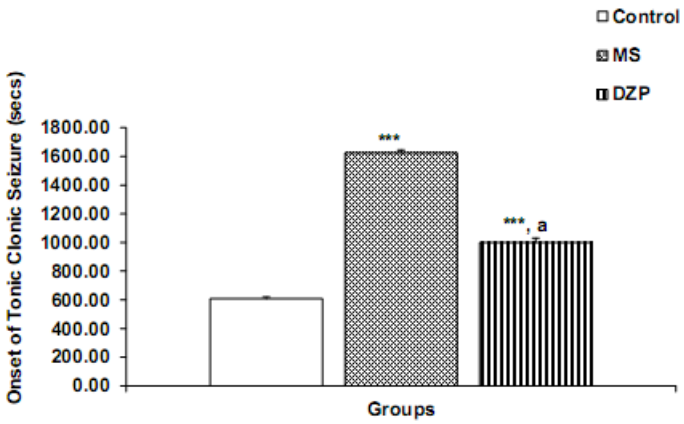

Fig-4: Comparison of onset of tonic clonic seizure in the different experimental groups

Values are mean ± SEM, n = 7.

***p<0.001 vs control; a = p<0.001 vs MS.

Time of Death

The mean values for the time of death for the control, MS treated, and DZP treated groups are 32.17 ± 2.73 per seconds, 130.50 ± 4.55 per seconds and 79.67± 2.86 per seconds respectively. The time of death was significantly (p<0.001) prolonged in the MS treated group compared with the control group. Also, the time of death was equally significantly (p<0.001) prolonged in the DZP treated group compared with control, but significantly (p<0.001) lower compared with the MS treated group (Figure 5).

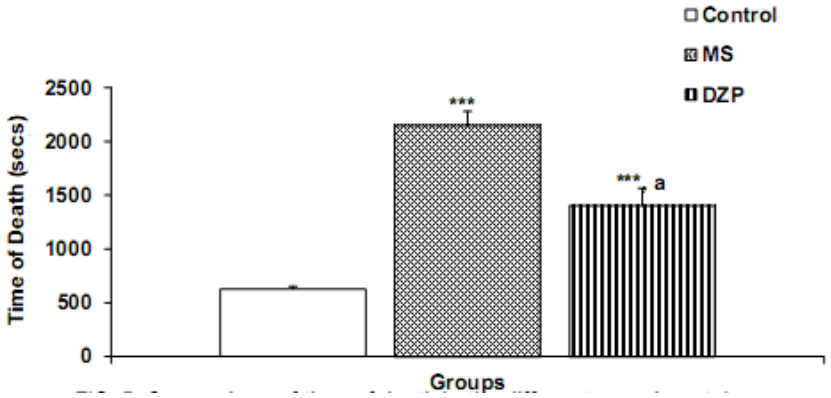

Fig-5: Comparison of time of death in the different experimental groups

Values are mean ± SEM, n = 7

***p<0.001 vs control; a = p<0.001 vs MS.

DISCUSSION

Seizure is a transient, spontaneous alteration of consciousness, behavior, motor activity, sensation, or autonomic function caused by a disproportionate rate and hyper synchrony of discharges from a group of cerebral neurons. A spontaneous recurrent seizures leads to convulsion and then epilepsy [20]. In our study, after the intraperitonial administration of 4-AP, the onset of trembling, wild running, tonic clonic seizures and time of death was initiated. However, after treatments with $MgSO_4$ and diazepam, the onset of trembling, wild running, tonic clonic seizures and time of death was delayed, connoting the anticonvulsive properties of these drugs. The onset of trembling, wild running, tonic clonic seizures and time of death was longer in the $MgSO_4$ treated group compared to the diazepam treated group suggesting that $MgSO_4$ is a better anticonvulsant following seizures induced by 4-AP.

Diazepam is one of the benzodiazepines in which its anticonvulsant effect is by the facilitation of the action of GABA [21]. Pena & Tapia [22] reported that 4-AP augments the release of glutamate and has been reported to encourage neurodegeneration and a simultaneous hyperactivation of the glutamate receptors leading to epileptiform discharges. Hence a facilitated GABA mediated transmission might oddly improve neuronal hyperexitation and seizures as a consequence, this can imaginably account for why diazepam could not curb seizures induced by 4-AP. Magnesium sulfate ($MgSO_4$) on the other hand is one of the conventionally used anticonvulsants even though its mechanism of action is not well understood [23].

However, as a potent vasodilator of cerebral vasculatures, it protects the blood brain barrier, decreased cerebral edema formation, improves cerebral arterial circulation and this has been reported to be a neuroprotective mechanism [15]**.** As an anticonvulsant, it exact mode of action has been reported to be through affecting sequence of cardiovascular and neurological functions and by altering calcium metabolism [24]. In our study, both diazepam and $MgSO_4$ prolonged the series of events that leads to the onset seizures induced by 4-AP but could not completely control it. This is in corroboration with a study by Yamaguchi & Rogawski [19], where it was reported that non-NMDA receptor mediated agonist like diazepam and $MgSO_4$ could not reverse seizures induced by potassium channel antagonists, rather NMDA mediated agonist such as phenytion and Carbamzapine were able to curtail seizures induced by 4-AP.

Conclusively, $MgSO_4$ and diazepam delayed the onset of events that led to seizures induced by 4-aminopyridine, but none could completely reverse the seizures condition, therefore the combined administration of 4-AP and either of these anticonvulsants may not offer any therapeutic benefit following seizures induced by 4-AP.

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