e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2024; 16(2); 1578-1583

Original Research Article

Anticonvulsant Effect of Ketamine in Electroshock and Chemo shock Seizure in Albino Rats

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Received: 25-11-2023 / Revised: 23-12-2023 / Accepted: 26-01-2024

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Conflict of interest: Nil

Abstract:

Background: In this study, we wanted to evaluate the anticonvulsant effect of ketamine in electroshock and chemo shock seizure in albino rats.

Methods: This was a Randomized Controlled Prospective study, parallel group designed, animal experimental study, where 42 male adult albino rats of age 7 months weighing about 150 to 200 gm were selected and conducted in the Institute of Pharmacology and Central Animal House attached Madurai Medical College, Madurai, for a period of nine months from January 2020.

Results: In MES study the latency phase time were noted. The mean duration of latent phase was control I 2.66±0.747, standard I 2.5±0.5, Test I 2.83±0.687. No latent phase in test II since no seizure occurred in test II. On comparing with the control there was a no significant difference in the duration of Latent phase between the standard I ($p < 0.05$) and test group I ($p < 0.05$) but a significant difference in the test II ($p < 0.0001$) Time taken from latent to THLE duration of control group I 24 ± 1.632, Standard group I 0, Test I ±7.5±0.9573 and Test II 0 respectively. There was complete abolition of extensor phase in standard I and test II. On comparing with the control there was a significant difference in the THLE between the standard I ($p < 0.00001$), test group I ($p < 0.00001$) and test II ($p < 0.0001$. The mean duration of latent phase after injection PTZ were control II 67.16± 2.114 standard II 0, Test III 106.66±3.944, Test IV 104±2.516. There was complete abolition of latent phase in control group II. On comparing with the control there was a significant difference in the duration of latent phase between the standard II ($p < 0.00001$), test group II ($p < 0.00001$) and test II ($p < 0.0001$).

Conclusion: More studies on ketamine on Refractory Status Epilepticus (RSE) and Super Refractory Status Epilepticus (SRSE) patient may be helpful to recognize ketamine as one of the first line drugs to treat Refractory Status Epilepticus (RSE) and Super Refractory Status Epilepticus (SRSE)

Keywords: Anticonvulsant, ketamine, electroshock, chemo shock, seizure, albino rats.

Introduction

The term "epilepsy" denotes any disorder characterized by recurrent unprovoked seizures. A seizure is a transient disturbance of cerebral function due to an abnormal paroxysmal neuronal discharge in the brain [1]. In central nervous system disorder, epilepsy is the 4th most common disorder. "THE DISEASE OF LIGHTENING" is the description about epilepsy by J. HJACKSON [2]. Till the15th century, the world believed that epilepsy was "the sacred" disease. In the 400 B. C. Hippocrates mentioned in his writing that epilepsy is a disease of the brain, advised to treat epilepsy by drugs, not by religious incantations. The Greek word Epilepsy means "TO SEIZE" or "TO TAKE HOLD OF" [3]. Many people with epilepsy feel stigmatized by the society and become isolated unnecessarily [4]. The international league against epilepsy 2005-2009, broadly classified it into focal seizures and generalized seizures. Common etiological factors of epilepsy include: metabolic disorder, electrolyte imbalance, acute or chronic effects of drugs, brain injuries due to hypoxemia, ischemia or trauma, space occupying lesions like tumors, infections, epilepsy in pregnancy and unknown cause. Total world incidence of epilepsy is ~0.3-0.5 % and prevalence is 5 to 30 persons per

one thousand people. [5] Wide range of drugs used to treat epilepsy vary from Valproic acid, Lamotrigine, Locasamide, Phenytoin, Carbamazepine, Topiramate, Zonisamide, Rufinamide, Gabapentine, Pregabalin, Phenobarbitone, Ethosuximide, Benzodiazepine, Taigabine, Retigabine, Parampanel etc.

Most of the drugs have to be given for long period, even lifelong. Chronic intake of drugs leads to mild side effects like sedation, rashes, tremors and severe adverse effects like ataxia, behavioral changes, blood dyscrasiasis, hepatotoxicity and various problems in neonates of known epileptic pregnant and lactating mothers. 5-6% of fetal abnormality has been estimated in children born to mothers with epilepsy who takes antiepileptic drugs. 20-30% of all patients of epilepsy have not been treated satisfactorily by the above drugs. Treatment with surgery, vagal nerve stimulation etc, have not given satisfactory results and have more of its own complications.

Epileptogenesis refers to the transformation of a normal brain into one that is chronically hyperexcitable. In Status Epilepticus, the seizures persist for a longer time to sustain the neurological injury. It is a life threatening emergency condition. If the Status Epilepticus condition continues for more than two hours and is not controlled by both first line and second line antiepileptic drugs (AED) it is called "REFRACTORY STATUS EPILEPTICUS" (RSE) [6].

The refractory status epilepticus is treated in intensive care units (ICU) with conventional anaesthetic agents like high dose Midazolam, Thiopentone sodium or Propofol. Some of the treatment failure cases have been reported for above drugs. Literature search for effective alternative drugs to treat RSE condition helped to identify another anaesthetic agent Ketamine. Hence planned to carry out an animal model study to evaluate anticonvulsive effect of Ketamine compared with standard Midazolam.

Aims and Objectives

1. To evaluate the anticonvulsant effect of Ketamine in Albino rats using
   - Maximum electro shock method (Electrical method)
   - Pentylenetetrazole method (Chemical method)
2. To compare the activity using the standard drug Midazolam.

Methods

This was a Randomized Controlled Prospective study, parallel group designed, animal experimental study, where 42 male adult albino rats of age 7 months weighing about 150 to 200 gm were selected and conducted in the Institute of Pharmacology and Central Animal House attached Madurai Medical College, Madurai, for a period of nine months from January 2020.

Methodology

The anti- epileptic property of Ketamine was evaluated using Maximum Electro Shock and Pentylenetetrazole methods.

Maximum electro shock method: Merrit and Putman (1938) showed the feasibility of testing various drugs and other chemical substances for potential clinical antiepileptic activity by means of simple electro shock method. In this method a 5- 10 times the threshold current (maximum electro shock) was used for inducing convulsions in rats which resembles generalized tonic clonic seizure in human. The phases of convulsions were observed viz, Tonic flexion. Extension, clonus, stupor and tonic hind limb extension (THLE). Agents which abolish the tonic phase of major seizures recognized as the agents have antiepileptic property [7].24 male adult albino rats were randomly grouped into four each containing six animals and given free access to food and water. On the day of the experiment the control group I received distilled water, the standard I, received Inj. Midazolam 5mg/kg intraperitonially (i.p.). Test group I & II received Inj. ketamine 50 mg/kgn and 100 mg/kg i.p. respectively.

Group	Category	Treatment
I	Control	Normal feed and water. MES
II	Standard I	Inj. Midazolam 5 mg/kg i.p., single dose 10 minutes before MES.
III	Test I	Inj. Ketamine 50 mg/kg i.p., single dose 30 minutes before MES
VI	Test II	Inj. Ketamine 100 mg/kg i.p., single dose 30 minutes before MES.

After every MES, Latent phase (time between MES and first occurrence of convulsion) and phases of convulsion with duration were observed THLE (tonic hind limb extension) was considered the end point of experiment and the result noted.

The result were tabulated and analyzed.

The MES Animals were observed for four weeks after the procedure to find out any change in behavioral, neurological, autonomic profile and mortality.

Pentylenetetrazole Method: It is a method introduced by Richard and Everett for inducing generalized convulsions by injecting the chemical Pentylenetetrazole. The phases of convulsion were observed. Dose is maximum 70 mg /kg.i.p.

Four weeks after MES test, 24 male adult albino rats were grouped into four each containing six animals and given free access to food and water. On the day of the experiment the control group II received distilled water the standard group II received Inj.midazolam5 mg/kg i.p. and test group III and IV received Inj. Ketamine 50 mg/kg and 100 mg/kg i.p. respectively.

Group	Category	Treatment
V	Control II	Normal feed and water. Chemoshock
VI	Standard II	Inj. Midazolam 5 mg/kg i.p., 10 minutes before chemoshock.
VII	Test III	Inj. Ketamine 50 mg/kg i.p., 30 minutes before chemoshock
VIII	Test IV	Inj. Ketamine 100 mg/kg i.p., 30 minutes before chemoshock.

The phases of convulsions induced by PTZ observed by its onset time and severity stages by Racine limbic seizure scale in10 stage version.

The stages are I) facial automatism, II) facial and head clonus, III) forelimb clonus, IV) forelimb clonus and rearing, V) Bilateral Forelimbs clonus with loss of posture, VI) multiple stages V seizure, VII) jumping, VII) running and jumping, IX) stage VIII followed by tonic seizures, X) multiple stage IX seizure The latent period and the occurrence of stage VI seizure were noted. The result were tabulated and analyzed. The Animals were observed for four weeks after the producer to find out any change in behavioral, neurological, autonomic profile and mortality.

Results

Table 1: Latent phase – MES method

Latent phase. Time in seconds				
Group Rat	**Control I**	**Standard I**	**Test I**	**Test II**
1	3	2	3	0
2	2	2	4	0
3	2	3	3	0
4	4	3	2	0
5	3	2	2	0
6	2	3	3	0
MEAN±SD	**2.66±0.747**	**2.5±0.5 ***	**2.83±0.687****	**0*****

* $p < 0.05$ ** $p < 0.05$ *** $p < 0.0001$. In MES study the latency phase time were noted. The mean duration of latent phase was control I 2.66±0.747, standard I 2.5±0.5, Test I 2.83±0.687. No latent phase in test II since no seizure occurred in test II. On comparing with the control there was a no significant difference in the duration of Latent phase between the standard I ($p < 0.05$) and test group I ($p < 0.05$) but a significant difference in the test II ($p < 0.0001$)

Table 2: THLE - MES method

THLE. Time in seconds				
Group Rat	**Control I**	**Std I**	**Test I**	**Test II**
1	23	0	8	0
2	22	0	7	0
3	27	0	9	0
4	25	0	7	0
5	23	0	6	0
6	24	0	8	0
MEAN±SD	**24±1.632**	**0 ***	**7.5±0.9573 ****	**0 ***

* $p < 0.00001$ ** $p < 0.00001$ *** $p < 0.00001$

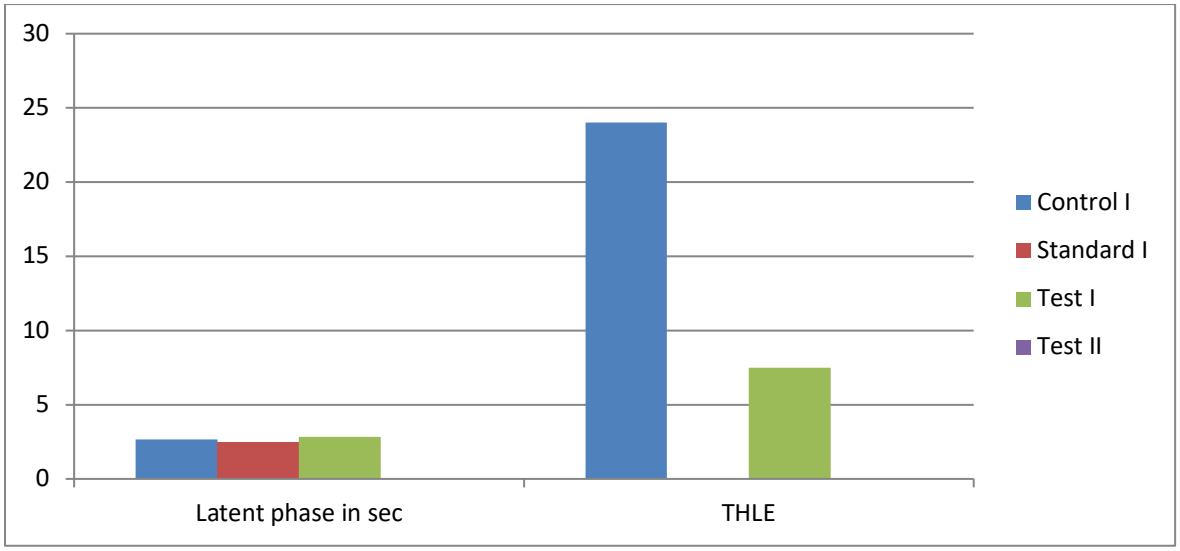

Figure 1: THLE - MES method

- MES Method (x-axis: groups / y-axis: duration in seconds): Time taken from latent to THLE duration of control group I 24 ± 1.632, Standard group I 0, Test I ±7.5±0.9573 and Test II 0 respectively. There was complete abolition of extensor phase in standard I and test II. On comparing with the control there was a significant difference in the THLE between the standard I ($p < 0.00001$), test group I ($p < 0.00001$) and test II ($p < 0.0001$)

Table 3: latent phase – PTZ

Latent phase. Time in seconds				
Group Rat	**Control II**	**Standard II**	**Test III**	**Test IV**
1	70	0	104	100
2	64	0	100	103
3	69	0	106	106
4	65	0	110	104
5	68	0	108	103
6	67	0	112	108
MEAN±SD	**67.16±2.114**	**0***	**106.66±3.944****	**104±2.516*****

* p <0.00001 ** p <0.00001 *** p <0.00001

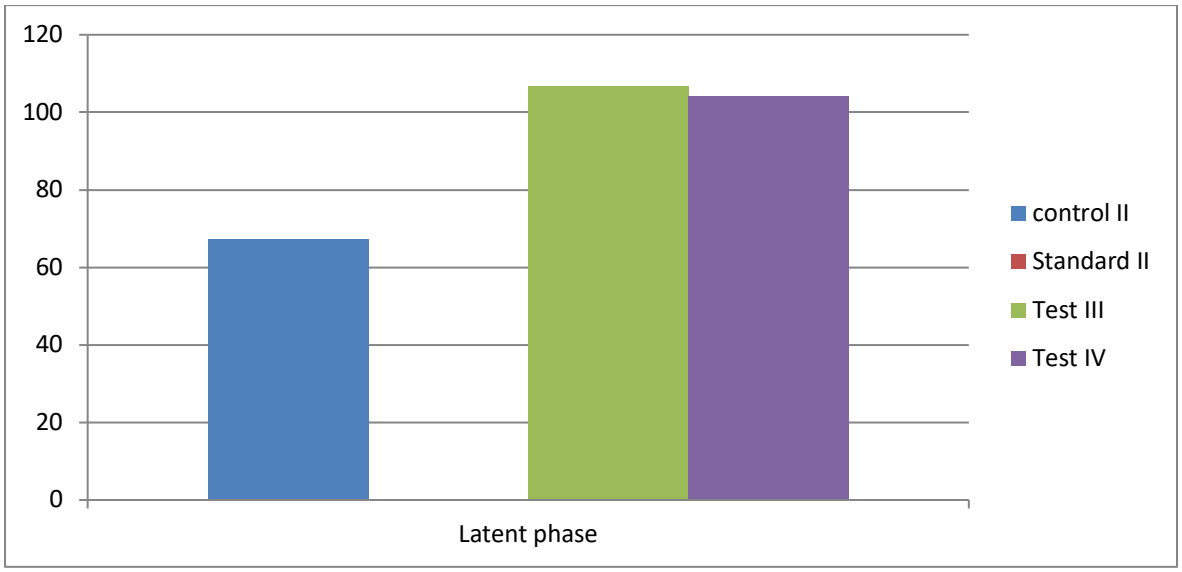

Figure 2: latent phase – PTZ

PTZ method (x-axis: groups / y- axis: duration in seconds)

Pentetylenetetrazole Method: The event of convulsions after PTZ and the response of test drug midzolam and ketemine observed. The stages of convulsions noted with time and duration in all groups.

Latent phase: The mean duration of latent phase after injection PTZ were control II 67.16± 2.114 standard II 0, Test III 106.66±3.944, Test IV 104±2.516.

There was complete abolition of latent phase in control group II. On comparing with the control there was a significant difference in the duration of latent phase between the standard II ($p < 0.00001$), test group II ($p < 0.00001$) and test II ($p < 0.0001$)

Table 4: Occurrence of seizure stage IV and above – PTZ method:

Group	Presence (yes)/ Absence (no)			
Rat	Control II	Standard II	Test III	Test IV
1	Yes	no	no	no
2	Yes	no	no	no
3	Yes	no	no	no
4	Yes	no	no	no
5	Yes	no	no	no
6	Yes	no	no	no

After latency phase the stages of convulsions observed in all groups and presence or absence of stage V with forelimb clonus with loss of posture noted, regaining from loss of posture and moving considered as control of epilepsy. But gaining posture and jumping, running and multiple seizure occurrence consider as uncontrolled epilepsy i.e. status epilepticus. Except control group II, all other groups didn't show the stage VI and above seizures.

Discussion

Epilepsies are common, heterogeneous by virtue of different seizure types, syndromes, causes, co morbidities, and other individual factors. Although 80%patients remain controlled with drug the rest continue to have seizures and their negative impact on quality of life, morbidity and risk of mortality. Surgery is effective in a small proportion of patients. As genomics and proteomics unfold, the cause of epilepsies will be better understood and helps in selecting optimum treatment. Epileptics need sympathetic well informed professional approach to integrate the science with personal life, pharmacological remedies, and holistic care. Condition like Status Epilepticus (SE) and Refractory Status Epilepticus(RSE), medical professional face great challenge to control it or reduce the duration of seizure, even lots of better new antiepileptic drugs are available at present. Uncontrolled or increased duration of seizure may cause irreversible neuronal damage to the individual and increase the chances of mortality and morbidity. Our study objective was to compare the anticonvulsant property of the ketamine with the standard drug midazolam, which is commonly used as antiepileptic by infusion in the condition like SE and RSE. Both Status epilepticus and Refractory Status Epilepticus commonly treated in intensive care units. In ICU monitoring of vitals and GCS (Glasgow Coma Scale), maintaining of the ventilation, and administration of drug through controlled tittered dose by infusion set are the better ways to support the progress of treatment. Seizure duration more than 30 minutes, 19% mortality may occur. 23-61% mortality rate in RSE and 90% relapse in survivors [8].

If initial management of status epilepticus (SE) with Inj Lorazepam, Phenytoin, Fosphenytoin, Valproate, Levitiracetam and Phenobarbital failed it is considered refractory status Epilepticus (RSE). It requires treatment with Anaesthetic agents like Midazolam, Propofol, Phenobarbitone and volatile anaesthetic gas. All the above agents cause hypotension as adverse effect which will leads the conditions worse if the RSE associated with septic shock, hypovolemic shock (e.g. Road traffic accident with blood loss and head trauma) requires multiple ionotropic agent support. Any patient with shock, maintaining blood pressure (BP) and mean arterial pressure (MAP) between 60 – 160mm/Hg is important to achieve cerebral perfusion, metabolic modification and healing of brain tissue. Failure rate of midazolam infusion is 14-18%58 Tachyphylaxis adverse effect of midazolam may not be helpful to give repeated dose in some cases [9].

The adverse effect of propofol like 'propofol-related infusion syndrome' associated with local systemic metabolic acidosis, rhabdomyolysis, lipemia, hyperkalemia and cardiac arrest may restrict its use beyond 48 hrs. Pentobarbital has adverse effects like bradycardia, poikilothermia and fruitability pulmonary infection i.e ventilator associated pneumonia (VAP) limits its use. Moreover its prolonged half-life 15-40 hrs may lead to delay in recovery of consciousness duration

12- 24 hrs, after stopping of infusion. 8 % acute failure and 8 % refractory hypotension noted in treatment of RSE with pentobarbital [10]. With the use of inhalational anaesthesia agents like Isoflurane or desflurane to treat RSE, epileptic discharges are adequately controlled. But it is needed to add combination therapy with injectable AED's during therapy. Volatile anaesthetic agents cause paralytic ileus as adverse effect. It's high cost, need of sophisticated delivery system and need of close monitoring has its own limitation.

Ketamine, a dissociate anaesthetic agent which is used as induction anaesthetic agent in hemodynamically unstable patient, Total intravenous anesthesia (TIVA) in the condition when the patient is not fit for general anaesthesia and spinal anaesthesia in selected cases, Day care surgical producers of pediatric and adult age group, intramuscular injection for apprehended preoperative children to sedate and put intravenous line. NMDA receptors are unregulated during SE/RSE/SRSE and contributes excitotoxicity may be countered by using ketamine, a NMDA receptor antagonist. Vasopressor sparing effect and neuroprotective effect of ketamine is more useful in shock patient with SE. It is cheap and readily available drug can be used as I.V. bolus or infusion as and when required. Its rapid onset of action and short duration of action facilitate the tittered dose delivery, very less withdrawal drug effect and may associate with or without common adverse effect of delirium. In recent studies, it was found that the sedation action of ketamine contributed by hyperpolarization-activated cyclic nucleotide (HCN1channels), which may be a favorable factor to control SE. The observation from Maximum Electro Shock method revealed that there was statistically decrease in the duration of latent phase and early occurrence of tonic hind limb extension (THLE) comparing standard with test I and II. The result concludes that ketamine has a protective effect against maximum electroshock induced convulsions which was comparable to the standard drug midazolam. The observation from the Pentylenetetrazole method has shown a statistically significant increase in latent phase (occurrence of first sign of seizure) and abolition of seizures above stage VI in test III and IV.

Conclusion

The injection ketamine possess good enough antiepileptic property against standard methods for evaluation namely the electrical methods and chemical methods to induce status epilepticus. Moreover ketamine does not cause hypotension, bronchospasm, respiratory depression and bradycardia in hemodynamically unstable patient. Hence more studies on ketamine on Refractory Status Epilepticus (RSE) and Super Refractory Status Epilepticus (SRSE) patient may be helpful to recognize ketamine as one of the first line drugs to treat Refractory Status Epilepticus (RSE) and Super Refractory Status Epilepticus (SRSE).

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