

SEIZURE ATTAINMENT AND MORTALITY OF MICE IN KAINATE- INDUCED STATUS EPILEPTICUS

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Received: 15 Apr 2013, Revised and Accepted: 03 May 2013

ABSTRACT

Objective: To evaluate two different methods of intraperitoneal (IP) administration and range of doses to establish optimal dose to induce status epilepticus (SE) in mice, the incidence of achieving stage 4 (S4) or above SE and mortality in kainic acid (KA)-induced SE.

Methods: KA was administered as bolus with dose range between 20 and 40 mg, and by intermittent injection of 25 mg and followed by 5 mg doses with 30-minute interval between doses. Each dose was tested on 3 mice. Following KA injections, mice were monitored continuously for the onset of SE, extent of seizure activity and mortality.

Results: All ICR mice displayed comparable seizures. Within eight minutes of the injection, mice were inactive accompanied by staring behaviour. This behaviour was followed by jerking, tail arching, head nodding, forelimb clonus, rearing, falling and generalised tonic-clonic seizure. Bolus dose of KA 30 mg/kg and above is sufficient to induce SE at S4 or above. Out of 18 mice, 8 reached S4 or above SE. For the 8 mice, 4 died from SE within 4 days depending on the dose given.

Conclusion: In mice, bolus dose of KA 30 mg/kg is sufficient to induce significant SE characteristics. Survival of the mice with S4 or above SE is up to 4 days depending on dose of KA given.

Keywords: Kainic acid, Status epilepticus, Seizure attainment

INTRODUCTION

Animal models are employed to enhance our understanding on epileptogenesis and the potential targets for anti-epileptogenesis therapies [1,2]. The animal chosen usually meets a determined taxonomic equivalence to humans and resembles human physiology. Two common animal models of epilepsy are kindling and status epilepticus (SE) [3]. SE model is technically easier to establish, but produces more variable in their expression of seizure manifestation. There are two currently known chemoconvulsants to produce epileptic seizure in this type of animal model; pilocarpine and kainic acid (KA). The chemoconvulsant model produces delayed appearance of spontaneous seizures or known as "post-SE models of temporal lobe epilepsy (TLE)" [2]. The morphological changes that occur in the hippocampus following SE are often similar to those seen in human TLE. Nevertheless the damage in the animal model can be more severe and widespread. KA is usually applied to adult rodents either via intraperitoneal (IP) or intracerebral. The systemic doses, varying between 8 to 12 mg/kg for rats and more than 20 mg/kg for mice result in sustained depolarization of neurons manifested by complex seizure behaviour [4]. Although systemic injection induces a convulsive SE with continuous clinical seizure activity, there are certain disadvantages, in which the status can be difficult to control, high mortality rate, the expression of spontaneous seizures can be unpredictable and the neural damage can be extensive [1].

Thus some researchers administer KA to the specific site of the brain such as the amygdala or the hippocampus [2]. It is reported that mortality is lower, but this procedure is labor intensive [2]. However, because of the specific injected site, morphology alterations are much more discrete and largely restricted to the ipsilateral hippocampus, no overt seizure activity was observed and spontaneous recurrent seizure (SRS) were typically only observed in electroencephalogram without any perceptible clinical manifestation [5]. As such, IP KA remains to be used in many studies. However, the profile of successes and mortality of this method in mice is limited.

The current study evaluated two different methods of IP administration and range of doses to establish optimal dose to induce SE in mice, the incidence of achieving stage 4 or above SE with established six points seizure scoring scale [6] and mortality in KA-induced SE.

METHODS

Research sample

Adult male ICR mice weighing 20-35 g were used in this study. The animals were kept in an animal room with controlled environment. The mice were grouped into polypropylene cages with 3 animals per cage. They were acclimatized for 1 week under hygienic conditions and maintained under standard laboratory conditions with 12 hour light/ 12 hour dark cycle and were allowed free access to standard pelleted mice food and water ad libitum. All experimental procedures in mice described were reviewed and approved by the Animal Ethics Committee of the university.

Kainic acid administration

Kainic acid (KA) (Sigma-Aldrich, USA) was dissolved in isotonic normal saline with final concentration of 1 mg/mL. Doses of 20 to 40 mg/kg of KA were administered intraperitoneally⁶. KA solutions were freshly prepared on the day of the experiment. The KA was administered as bolus with doses of 20 mg, 25 mg, 30 mg or 40 mg and by intermittent injection of 25 mg + 5 mg and 25 mg + 5 mg + 5 mg doses with 30-minute interval between doses. Each dose was tested on 3 mice.

Seizure Attainment

Following KA injections, mice were placed in clear plastic cages and monitored continuously for three hours for the onset and extent of seizure activity. The mice were scored and evaluated using a defined seizure scoring scale by Schauwecker and Steward (1997) [6]; stage 1 (S1), immobility (unmoving and crouched in a corner); stage 2 (S2), forelimb and/or tail extension, rigid posture (stretches body out, tail becomes straight & rigid, ears laid back, bulging eyes); stage 3 (S3), repetitive movements, head bobbing; (repetitive head bobbing, rears into a sitting position with forepaws resting on belly); stage 4 (S4), rearing and falling (tonic-clonic seizures broken by periods of total stillness, jumping clonus, running clonus); stage 5 (S5), continuous rearing and falling; (continuous level 4 seizures) and stage 6 (S6), severe tonic-clonic seizures; (body in clonus, no longer using limbs to maintain posture usually precursor to death). Seizure parameters monitored include onset of seizure, latency of convulsion, duration of seizure activity and mortality.

RESULTS

Data from this study was obtained from a total of 18 mice. All mice displayed comparable seizures. Within eight minutes of the injection, mice were inactive accompanied by staring behaviour. This behaviour was followed by jerking, tail arching, head

nodding, forelimb clonus, rearing, falling and generalised tonic-clonic seizure. Out of 18 mice, 8 reached S4 or above according to the Schauwecker & Steward (1997) seizure scoring scale⁶ (Table 1). From 8 mice which reached S4 and above, 4 died from SE. Detailed analysis of all the mice reaching S4 and above is shown in Table 2.

Table 1: Effect of kainic acid administration on seizure parameters

Technique	Kainic acid (mg/kg)	n	Seizure incidence at least Stage 1 ^a	Mean onset of Stage 1 seizure (min) ^{a,b}	No. of mice achieving Stage 4 or above seizure ^a
Single bolus	20	3	3/3	15.3±11.8	1/3
	25	3	2/3	30.3±38.6	1/3
	30	3	3/3	29.0±4.6	3/3
	40	3	3/3	8.3±1.7	2/3
Intermittent	25+5	3	3/3	8.3±1.7	1/3
	25+5+5	3	3/3	21.7±16.4	0/3

a. Based on Schauwecker & Steward (1997).

b. Onset to achieve at least stage 1 seizure from the 1st injection.

Table 2: Mean duration achieving stage 4 or above of status epilepticus and mortality after kainic acid administration

Kainic acid (mg/kg)	n	Mean onset to Stage 4 or above (min) ^{a,b}	Mean duration of survival ^b	Mortality
20	1	52.0	-	0/1
25	1	11.0	53 mins	1/1
30	3	41.0	4 days	1/3
40	2	27.0	3 days	2/2
25+5	1	40.0	-	0/1

a. Based on Schauwecker & Steward (1997).

b. Time between onset of Stage 4 and above and death.

DISCUSSION

KA-induced seizures are widely used as a TLE model due to its behavioural and pathological similarities [7]. Studies have shown that systemic injections of KA cause epileptiform seizures in the CA3 region of the hippocampus. These seizures propagate to other limbic structures and are followed by a pattern of cell loss similar to that seen in TLE patients [7]. While KA-induced neuronal injury is well-characterized in rats, relatively little data is available on the use of KA and its consequences in mice. The current study therefore examined the KA dose-response relationship and behavioural changes in ICR mice, an outbred strain of mice. Eight of the tested mice exhibited repetitive, tonic-clonic seizures. Based on previous study, these mice should have developed hippocampal neurodegeneration [7]. Death usually occurs during a violent tonic-clonic seizure, associated with breathing failure. KA model clearly fulfils the criteria with regards to human TLE since the hippocampus, amygdala and other limbic structures play a central role in symptomatology, patterns of brain damage is clearly reminiscent of Ammon's horn sclerosis with a similar gradient of vulnerability, spontaneous seizures with a limbic symptomatology are consistently noted following parenteral and intracerebral KA, and available anticonvulsants are weak against the seizures generated by KA [7].

KA sensitivity and mortality

The most widely used mouse and rat strains in animal models are C57BL/6 mice, BALB/c mice, Sprague-Dawley rats, and Wistar rats. Other strains, such as A/J mice, CD-1 mice, and ICR mice, were also used. While both C57BL/6 and BALB/c mice are strains inbred to establish the genetic homogeneity, CD-1 and ICR mice stand out among the most commonly used research mice as an outbred stock to minimize genetic change [8]. Certain strains of mice are strongly resistant to seizure-induced excitotoxic cell death in spite of similar seizure severity, but in general ICR strains were resistant to seizure [9]. Mouse model offer an attractive strategy for investigating complex neurological disorders, besides affordable and low body weight. The majority of genetic studies have employed mice, not only because their genomes are relatively similar to that of human, and more completely characterized [10], ease of handling, high

reproductive rates, and availability of extensive sequence data for inbred strains [11].

The doses tested had very different thresholds for seizure activity, seizure behaviour, and dose tolerance in response to KA injection. There are several published studies using male ICR mice to induce epilepsy with 25 mg/kg [12], 30 mg/kg [13,14] and 45 mg/kg [15] of KA.

Around 44% of mice reached SE at S4 and above; among which 50% of these mice died after SE manifestation. McLin & Steward (2006) found that mortality for ICR mice due to KA was 20-30%, but there are other strains such as C57BL and BALBc mice which were particularly vulnerable to seizure-induced death (40-50% mortality) [9]. The difference in the findings may be due to the different study protocol. Several studies have recommended the use of diazepam to reduce mortality rates. Diazepam effectively reduced the mortality (to less than 18%) [16] and did not affect the severity of histopathology of the brain of the mice with SE¹⁷. Nevertheless, diazepam intervention was not employed in the current study.

Seizure behaviour

KA initiates convulsive behaviour that persists for hours after a single injection. Currently, there is no universal approach to identify and classify seizures in animals, by electrophysiology or behaviour. Definitions of experimental seizures are numerous and more restrictive than those used for humans [18]. In the current study, the Schauwecker & Steward (1997) seizure scoring scale was used to categorize the epilepsy stages [6]. Most of the mice were inactive with 'staring' spells within 30 minutes of KA injection. These were followed by numerous wet dog shakes. The clinical signs showed are somewhat similar to the finding of Ben Ari (1985) [7]. The second phase (1 hour) was occurrence of individual recurrent limbic motor seizures as they became progressively more complex and prolonged. Between 1 - 2 hours, full status epilepticus appeared. This can be referred to Ben Ari's limbic motor syndrome [7]. In this experiment, mean duration for mice to reach S4 and above started as early as 11 minutes. Our findings showed that early onset SE (< 30 minutes) were associated with high mortality.

Comparison between single and multiple KA injection protocols

Administration via bolus injection produced high mortality rate during [19] or after the treatment [20,21] and low percentage of rodents becoming epileptics [22]. Some studies were conducted to avoid such situations by giving repeated low dose of KA [1]. Repeated doses of kainate (5 mg/kg per hour) were administered to develop S3 to S5 for 3 hours or more. This treatment protocol had a relatively low mortality rate (15%) [23]. Adapting similar repeated doses of KA for mice, the current study found that all mice reached S1 seizure and only one mice reached S4 or above. This may suggest that bolus dosing is more effective in inducing SE in mice than repeated low doses.

As conclusion, KA-induced seizure is known to resemble human TLE but there are limited data on dose-response relationship in mice. In mice, bolus dose of KA 30 mg/kg is sufficient to induce significant SE characteristics. Survival of the mice with stage 4 or above SE is up to 4 days depending on dose of KA given.

ACKNOWLEDGEMENTS

We are grateful to Universiti Kebangsaan Malaysia for the support under the UKM-GGPM-TKP-062-2010 grant.

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