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Levetiracetam prophylaxis ameliorates seizure epileptogenesis after fluid percussion injury

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ABSTRACT

To determine whether post-traumatic seizure severity would be affected by the interval between seizures and head injury, we measured seizures after various times with or without fluid percussion brain injury (2 atm fluid percussion injury; FPI). To determine efficacy of anti-seizure medication, we also determined if levetiracetam (LEV) would alter the relationship between injury and subsequent seizures. Early post-traumatic seizures were induced by Kainic acid (KA) at one week after 2 atm fluid percussion injury (FPI) in one group (FPI-ES). Seizures were induced at two weeks after FPI by KA in another group (FPI-LS). In addition, one group had induced seizures by KA without FPI, (sham-ES). Finally one group of animals received the antiepileptic agent (levetiracetam) infusion for one week after FPI and then had seizures induced by KA (FPI-LEV-ES). We measured seizure onset time, ictal duration and severity of seizures using a modified Racine's scale. Histopathological changes in the hippocampus CA1 region were also analyzed. Severity of seizures were increased in the FPI-ES group compared with sham-ES animals. Severity was also enhanced in early post-injury seizures induced by KA (FPI-ES vs. FPI-LS); this exacerbation of seizure severity could be ameliorated by levetiracetam infusion (FPI-ES vs. FPI-LEV-ES). Neuronal degeneration in CA1 was more severe in the FPI-ES group and this degeneration was also diminished by LEV. We conclude that early post injury seizures exacerbate susceptibility and severity of post traumatic seizures and increase neuronal degeneration in the CA1 layer of hippocampus. These changes are partially reversed by LEV infusion after FPI.

1. Introduction

Seizures subsequent to head injury are one of the most serious sequelae of such injuries and significantly impacts on the daily life of patients and their families. Post-traumatic epilepsy (PTE) is defined as a recurrent seizure disorder due to injury to the brain following trauma (Agrawal et al., 2006).

Seizure generation is related to development of abnormal seizure foci, malfunction of inhibitory neuron networks and pathological excitatory network connections. “Epileptogenesis” refers to a transformative process within the brain (Lothman et al., 1991); this process typically involves structural alterations in neural circuitry which may result from progressive neuronal damage and/or “self-repair” mechanisms within a latent variable period and culminates with the emergence of spontaneous recurrent seizures (Dudek and Spitz, 1997). That this process includes a latent period of variable time suggests that a progressive series of cellular changes may be involved.

Seizures after traumatic brain injury are clinically classified based on the time of their occurrence after the injury: immediate or injury-associated (<24 h after injury), early (<1 week after injury), and late (>1 week after injury) (Agrawal et al., 2006; Haltiner et al., 1997); this classification scheme is thought to represent different pathophysiological processes(Agrawal et al., 2006; Semah et al., 1998).

Understanding the epileptogenic process after TBI should help elucidate the importance of these cellular mechanisms in PTE and allow development of new therapeutic targets. An important goal of studies which investigate post-traumatic epileptogenesis is to

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dissociate injury-induced cellular alterations that promote seizure generation from compensatory and/or "self-repair" responses (Hunt et al., 2013).

In this study, to determine if time between brain injury and seizure occurrence may affect epileptogenesis, we studied susceptibility and severity of seizures with or without brain injury (2 atm fluid percussion injury) and also compared these paradigms between early (FPI-ES) and late (FPI-LS) post-trauma group KA-induced seizures. We induced seizures by kainic acid (KA) injection at one week post fluid percussion injury (FPI) in one group to mimic early post injury seizures and induced seizures 2 weeks post FPI in another group to mimic late post-injury seizures. We also studied if prophylactic levetiracetam infusion for one week after FPI could ameliorate seizure severity.

2. Material and methods

2.1. Ethics Statement

Male Sprague-Dawley rats (n=40), 6 weeks of age, were used in the present study. Animals were housed under a 12 h light/dark cycle, and provided with food and water ad libitum. All animal procedures were conducted in accordance with NIH guidelines reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the National Defense Medical Center (Taiwan Protocol Number IACUC-13–145). The number of animals used was the minimum number based on power calculations (α=0.05 and 1-ß=0.80). All surgery was done under aseptic conditions and postoperative analgesia was provided by administration of NSAIDs. We could not use opiates postoperatively as they have been shown to be neuroprotective which would confound interpretation of the results.

2.2. Experimental protocols and groups of animals

Kainic Acid (Sigma-Aldrich, Co., St Louis , MO, US, 7 mg/kg) tail vein injections for seizure induction in early stage groups (FPI-ES group) were started at one week after FPI and then at 2 and 3 weeks after injury. The seizures in the late post injury groups (FPI-LS) were induced by KA tail vein injection at two, three, and four weeks after FPI to insure equal numbers of seizures in both groups. A third group received KA-induced seizures but no FPI sham-ES. All animals received electrode implantation into the M1 cortex and EEG and video recordings were performed simultaneously (Fig. 1). To control for postictal apnea, all animals received manual artificial respiration by a tubing over the nose until spontaneous respiration was seen.

2.3. Fluid percussion traumatic brain injury

The fluid percussion device (model HPD-1700, Dragonfly R&D, USA) used to produce TBIs in rats has been described by Matsushita et al. (2000). The specific methods to produce fluid percussion injury are detailed in our previous papers (Chen et al., 2014; Eakin et al., 2013) but are reiterated here to facilitate reproducibility by other investigators. All rats were surgically prepared for midline FPI (mFPI). Briefly, animals were anesthetized using Zoletil 50 mg/kg IP; a 4.8 mm diameter burr hole was produced midline between the coronal and lambdoid sutures, and a Leur-Loc hub was affixed to the perimeter of the burr hole using cyanoacrylate. Dental acrylic and two small nickel-plated screws were used to anchor the hub to the skull. Twenty-four hours later, at the time of injury, the rats were re-anesthetized, the surgical

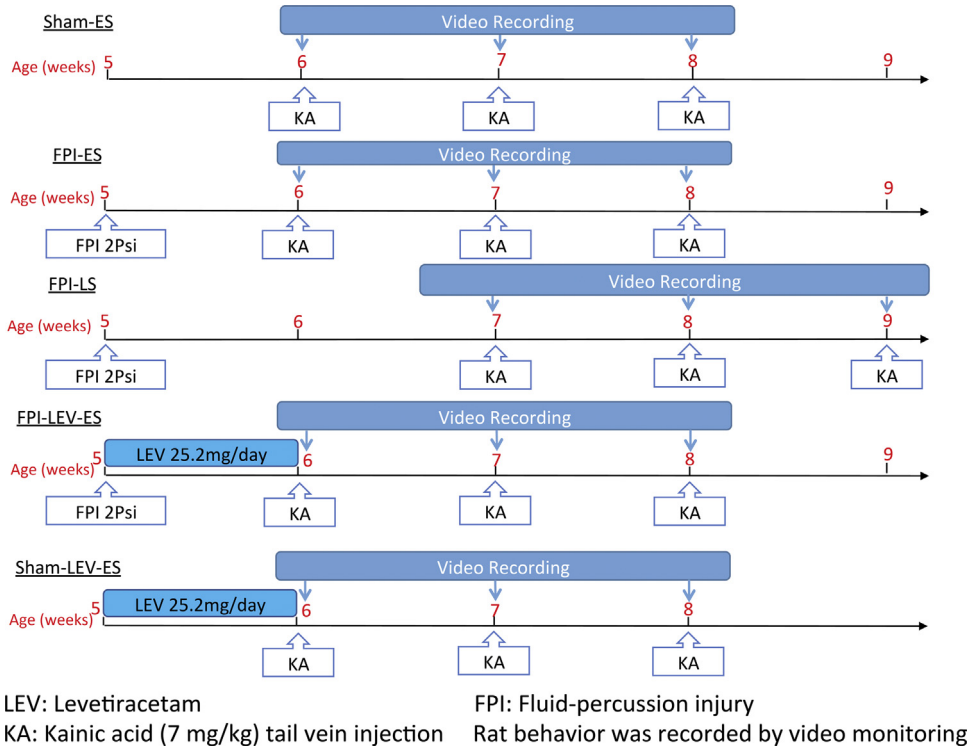

Fig. 1. The experimental protocol and groups of animals. Two groups of animals received 2 atm fluid percussion injury (FPI) or sham surgery (sham group) at 5 weeks old. Then both groups received the KA (7 mg/kg) tail vein injections one week after injury and subsequently 2 and 3 weeks after injury for seizure induction; they are referred to as the sham group (group1: sham-ES) and early seizure group (group 2 : FPI-ES group). The 3rd group are animals that received KA-induced seizures at 2, 3 and 4 weeks after 2 atm-FPI, referred to as the late seizure group (group3: FPI-LS). The 4th group are FPI animals receiving LEV pump infusion therapy for one week after injury and then received KA-induced seizures at one, two and three weeks after the injury, which is referred to as the prevention group (group 4 : FPI-LEV-ES). All of the animals received electrode implantation.