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Age-dependent mortality in the pilocarpine model of status epilepticus

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ABSTRACT

Status epilepticus (SE) is an acute neurological emergency associated with significant morbidity and mortality. Age has been shown to be a critical factor in determining outcome after SE. Understanding the causes of this increased mortality with aging by developing an animal model to study this condition would play a major role in studying mechanisms to limit the mortality due to SE. Here we employed pilocarpine to induce SE in rats aged between 5 and 28 weeks. Similar to clinical studies in man, we observed that age was a significant predictor of mortality following SE. While no deaths were observed in 5-week-old animals, mortality due to SE increased progressively with age and reached 90% in 28-week-old animals. There was no correlation between the age of animals and severity of SE. With increasing age mortality occurred earlier after the onset of SE. These results indicate that pilocarpine-induced SE in the rat provides a useful model to study age-dependent SE-induced mortality and indicates the importance of using animal models to elucidate the mechanisms contributing to SE-induced mortality and the development of novel therapeutic interventions to prevent SE-induced death.

Status epilepticus (SE) is a major neurological emergency associated with significant morbidity and mortality [7,8,11]. Clinical studies from our group and others have demonstrated that SE-induced mortality occurs as the result of the effects of prolonged seizures on the body [15,27]. Human data has demonstrated a role for hemodynamic and cardiac factors in the precipitation of death following SE [2] and that age is a critical factor in determining the outcome after SE [16,26,27]. Clinical studies have shown that following SE, the adult population has a much higher mortality than the younger population [27]. Thus, it is important to develop animal models that can be used to elucidate mechanisms underlying age-dependent mortality following SE and to develop potential therapeutic strategies to prevent death.

This study evaluated the effects of age as a contributing factor to the mortality following pilocarpine-induced SE in the rat and determined if SE in animals manifests an age dependency as observed in the human. Our results show that age is a significant predictor of mortality following SE. This model could be extremely useful for deciphering the pathophysiological mechanisms underlying SE-induced mortality in man and highlights the need to develop animal models to study mortality from SE.

All animal use procedures were in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by Virginia Commonwealth University's Institutional Animal Care and Use Committee. Animal procedures were carried out in a manner to minimize any pain and suffering. Male Sprague–Dawley rats (Harlan, Indianapolis, IN) ranging in age from 5- to 28-weeks were housed two per cage on a 12-h light/dark cycle and provided with food and water *ad libitum*. All animals were handled identically and provided with enrichment materials using standard procedures in our animal care facility. The results presented in this study were obtained by a retrospective analysis of data obtained over a 4-year period with a pooled $n = 218$ animals to evaluate the incidence of age-dependent mortality due to SE. The widely used pilocarpine-induced SE model in rat was employed using previously established methods in our laboratory [9]. Pilocarpine is a chemoconvulsant that initiates seizures [28] via activation of muscarinic M1 receptors [10]. Upon induction of seizures, their maintenance is dependent upon other mechanisms; mainly activation of the NMDA receptor by release of the excitatory transmitter glutamate [23]. To minimize peripheral effects of M1 receptor activation, rats were first administered methylscopolamine (1 mg/kg, i.p.) (Sigma, St. Louis, MO, USA) 30 min before pilocarpine (375 mg/kg, i.p.). Onset of SE was determined by the presence of continuous class 4–5 level seizures as assessed

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using a modified Racine scale [20]. Racine scale assessments were objectively evaluated by a minimum of three trained observers. Each observer independently scored the SE and following the experiment the scores were averaged for each animal. Thus, each final Racine score for an individual animal was based on the average of a minimum of three observations. Surviving animals undergoing SE for a duration of 60 min were rescued by administration of three consecutive injections of diazepam (5 mg/kg, i.p.) (VCU Health Systems Pharmacy, Richmond, VA, USA) at 1, 3 and 5 h following SE onset to terminate seizures. No major differences were noted to diazepam response between age groups used in our study. Animals were constantly observed for mortality. Surviving animals were supplemented with saline and lactose solution and were transferred to cages and returned to the vivarium and underwent continued observation for mortality. We used at least 10 animals per age group. Data were expressed as percent mortality. Significance was tested following a multi regression analysis employing a pair-wise comparison with a Wald Chi-square test using SAS 9.1.3 (Statistical Analysis Software, Cary, NC) and was plotted using Sigmaplot 8.0 (SPSS, Chicago, IL). Survival curves were examined using Kaplan–Meier Survival Analysis.

We evaluated the effects of pilocarpine-induced SE in animals at 5, 7, 10, 15, 18 and 28 weeks of age. The animals were monitored for seizure severity (Racine score and electrophysiology) and for mortality during SE for up to 48-h following onset of SE (Fig. 1 and Table 1). Forty-eight hours following onset of SE is a time point whereby SE-induced mortality has reached a plateau in all age groups studied under our experimental conditions. We choose 5-weeks as our first age group because the validity of modeling SE in rats in the first 2 weeks after birth is questionable due to the immature hypothalamo–pituitary–adrenal axis [21]. Thus, the 5-week time point allowed for full maturation of the stress system and also represents a time point before puberty is achieved in rats. The 28-week time point was an age where the rat is adult by human standards and we observed maximal mortality with this model of SE. The selected dose of pilocarpine (375 mg/kg, i.p.) is commonly used in our laboratory. Previous studies have evaluated different doses of pilocarpine and demonstrated that pilocarpine at 375 mg/kg can evoke maximal electrographic seizure activity during SE (reviewed in [5]).

Electrophysiological studies were conducted using standard procedures [22]. Animals were monitored throughout the SE using a video EEG monitor (BMSI 5000, Nicolet) and spike frequency and pattern characterized using Insight II (Persyst Development Corporation). Spike frequency was recorded using 6–18 s epochs. During each pattern spike frequency was quantified by measuring an average for each channel. Final results were then averaged for each postnatal age. Animals were allowed to progress for at least 20 min to ensure good SE recording. In agreement with our previous studies [3,18,19,22], electrographic analysis of pilocarpine-induced seizure activity in rats revealed that there was no significant difference in average time from first seizure to SE with age. The electrographic spike patterns (Fig. 1A) were not different for animals in different age groups during SE (5-, 15- and 28-week). Spike frequency during SE did not increase across the age groups used in our study. The elec-

Table 1
Electrographic spike frequency and behavioral Racine scores for different age groups of rats following pilocarpine-induced SE.

Age (weeks)	Spike frequency (Hz)	Racine score (mean ± S.E.M.)
5	7.85 ± 0.61	5.0 ± 0.0
7	9.02 ± 0.93	3.93 ± 0.07
10	9.08 ± 0.10	4.0 ± 0.0
15	9.07 ± 0.42	3.8 ± 0.06
18	8.85 ± 0.72	5.2 ± 0.08
28	7.95 ± 0.80	6.0 ± 0.0

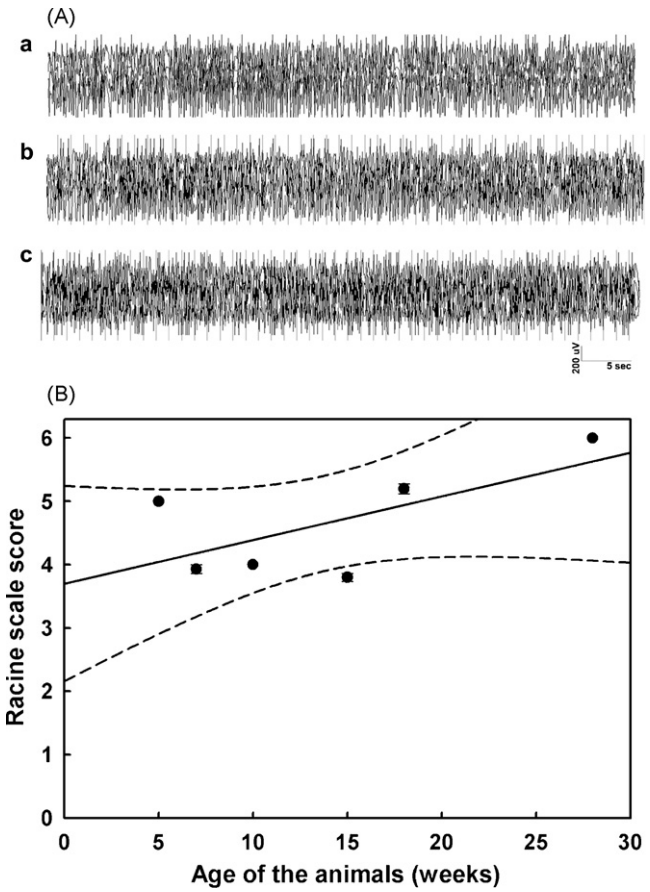

Fig. 1. Severity of SE following pilocarpine administration in animals of different ages. (A) Electrographical traces were obtained and analyzed. No significant differences in the spike frequency or EEG pattern were observed for (a) 5-week, (b) 15-week and (c) 28-week-old animal. (B) The severity of pilocarpine-induced seizures was quantified using a modified Racine scale (0: no response to 6: death) by averaging scores from at least 3 *independent* observers (mean ± S.E.M.). Linear regression analysis of seizure severity and age revealed that there was no correlation between Racine scale score and animal age ($r^2 = 0.43$) ($n = 10$, 29, 69, 90, 10 and 10 animals for 5, 7, 10, 15, 18 and 28-week age group, respectively).

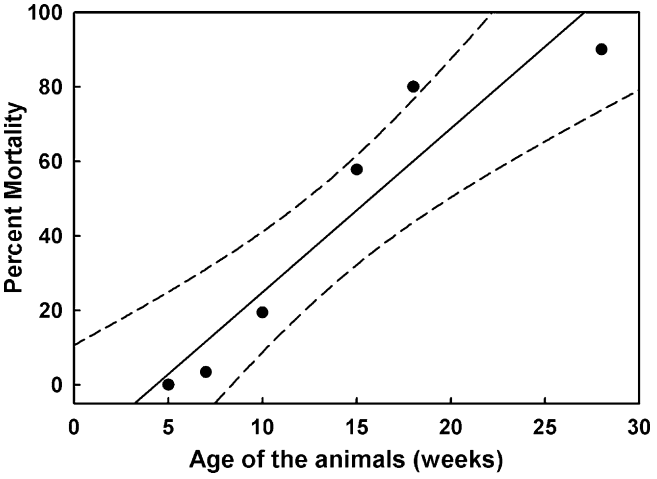

Fig. 2. Age-dependent mortality in the pilocarpine model of SE. Deaths due to SE increased progressively with age and development. Linear regression analysis gave $r^2 = 0.89$. Dotted lines represent 95% confidence interval limits.