ASSESSMENT OF XXX EFFECTS ON RECURRENT SEIZURE ACTIVITY IN THE RAT PILOCARPINE MODEL

6 May 2015

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This study was conducted between X 2015 and Y 2015 by:

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1. Introduction

Over 1 in 5,000 people each year in industrialized countries are diagnosed with status epilepticus (SE) [1-3], a continuous or serial seizure lasting 30 minutes or longer [4]. Of those that survive SE, nearly a third will develop spontaneous recurrent seizures, or epilepsy [5-7]. This form of acquired epilepsy is often refractory [6] and so its victims face lifelong hardships and financial burdens which extend to their communities [8]. Since SE-induced epilepsy is difficult to treat, an ideal therapy would prevent the development of epilepsy. However, no such therapy has yet been found [9, 10].

This study was conducted to determine the disease modification potential of XXX; specifically, to determine the effect of XXX administration on the development of, and degree of severity of, seizure activity following induction of status epilepticus in the rat.

2. Methodology

Animals

A total of 26 adult male Sprague-Dawley rats (90 days of age at initiation of study, weight range 329-400 g) were obtained from Harlan, Inc. (Indianapolis, IN) and were used as test subjects. Animals were allowed to acclimate to the holding facility for one week before study initiation. For the study, rats were initially divided randomly into two groups: A) status epilepticus (SE) plus administration of XXX (10mg/kg), and B) status epilepticus plus administration of drug-vehicle (30% HPBC). Complete data sets were obtained from 6 animals in the first group (SE + XXX) and 5 animals in the second group (SE + vehicle). Six animals in the first group and seven animals in the second group were non-responders (animals that received pilocarpine, but did not develop SE). In addition seven animals did not survive the SE event, dying within 24 hours of SE induction (5 vehicle and 2 drug-treated animals).

Compound Preparation

Drug vehicle was prepared by dissolving 15g hydroxypropyl-β-cyclodextrin (HPBC) into 39.5 mls dH2O. XXX (200 mg) was dissolved into 18 mls HPBC, plus 600 µl 1N HCL and sonicated until dissolved. The pH was adjusted using 1N NaOH, with final volume adjusted to 20 mls using dH2O.

Status Epilepticus

[3]
SE was induced as routinely performed in this laboratory [11, 12], with slight modifications. Approximately 30 minutes prior to seizure induction, all animals were treated with methylscopolamine (1 mg/kg, ip Sigma) to reduce peripheral effects of pilocarpine treatment. To initiate seizure activity, animals were administered pilocarpine nitrate (375 mg/kg, ip Sigma). Except for the test compound and diazepam, all drugs were dissolved in sterile saline. To facilitate solubility, pilocarpine solutions were warmed and sonicated. Diazepam was pharmaceutical grade and used without dilution, at 5 mg/kg ip.

**Drug Administration**

Following pilocarpine administration, animals were observed for overt ictal activity. T=0 was defined as onset of first discernible seizure. SE was defined as the onset of seizure activity without a clear offset (i.e. SE), which occurred approximately 10 min after observation of initial discrete seizure activity. XXX (10 mg/kg) or vehicle control was administered at t=40 minutes (i.e. 30 minutes after the onset of SE). Three doses of diazepam (5mg/kg each) were administered at t=70 min, and t = 3 hrs and 5 hrs to all animals. XXX or vehicle control was administered daily (1x/day) for 4 days (5 treatments total) by oral gavage, except for the initial and second dose which were administered i.p.

**Behavioural Monitoring**

All animals were allowed to recover from the SE event for 21 days, which allowed for the development of recurrent seizure activity [13]. After this period, monitoring began using videotape recording to assess seizure expression (i.e., Day 1 of recording = Day 22 of the study). To reduce sampling bias, animals were randomly selected for recording until a total of at least 40 hours were obtained for each rat (Table 1). Rats were recorded over two 2-day periods, with approximately 8 hours being acquired on the first day and approximately 12 hours acquired on the second day of each pair. The following table illustrates the recording schedule for all animals. (Rat#2 [Vehicle] was recorded for a further 20 hours over two days, and Rat Z [No-SE] was recorded for an extra 8 hours on one further day, both due to errors in animal selection.
Table 1. Recording schedule for all rats. First data column is the individual rat designation (number or letter)

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<th>Recording Day</th>
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Any observed seizures were assessed for duration, severity (Racine score – Table 2) [14], and number observed. Normality of the obtained data distribution was determined by Shapiro Wilk test (α=0.05). All data passed normalcy, and were analyzed using Student’s t test. Data are presented as mean ± SEM.

No animals needed to be sacrificed during the course of this study.

Table 2. Racine Scale to assess seizure severity

<table>
<thead>
<tr>
<th>Racine Score</th>
<th>Behavior</th>
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<tbody>
<tr>
<td>0</td>
<td>Normal Behavior, no paroxysms</td>
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<tr>
<td>1</td>
<td>Facial twitching, whisker bristling</td>
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<tr>
<td>2</td>
<td>Head Bobbing</td>
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<tr>
<td>3</td>
<td>Forelimb Clonus</td>
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<tr>
<td>4</td>
<td>Rearing + forelimb Clonus</td>
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<tr>
<td>5</td>
<td>Rearing and Falling Backward</td>
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</tbody>
</table>
3. Results

a. Effect of XXX on the development of recurrent seizure activity.

As expected, all animals in the vehicle group that developed status epilepticus also developed recurrent seizure activity (100% response; 5 of 5 animals) (Figure 1). However, administration of XXX reduced the probability of developing recurrent seizure activity by 50%. Recurrent seizure activity was observed in 3 of 6 XXX-treated animals. In one of those animals, there was only a single ictal event.

No recurrent seizure activity was observed in animals that received pilocarpine, but did not develop status epilepticus (non-Responder Group).

In animals that did display recurrent seizure activity, administration of XXX did not significantly reduce the average number of seizures observed. The average number of seizures observed in vehicle –treated animals was 5.4 ± 2.3 (sem) seizures compared to 3.3 ± 2.1 (sem) seizures in drug-treated animals. This difference was not significant (p =0.329, Mann-Whitney Test). However, these data are skewed by the fact that two drug-treated animals expressed several seizures whereas three drug-treated animals did not display any recurrent seizure activity. The median seizure expression was 0.5 seizures/animal (range 0-12) in XXX-treated animals, compared to a median seizure expression of 5.0 seizures/animal (range 3-9) in vehicle-treated animals. Taken together these data suggest that XXX reduced overall recurrent seizure activity when compared to SE vehicle treated animals.

b. Effect of XXX on seizure severity in animals that did develop recurrent seizure activity.

Administration of XXX modulated seizure characteristics when compared to vehicle-treated animals. In particular, seizures observed in vehicle-treated animals often developed from sleep, displayed a complex behavioral pattern that began by forelimb clonus, rearing and falling, whole body tics as well as wet dog shakes and facial automatisms (see attached video). Average seizure duration was 87.0 ± 6.8 (sem) seconds. Seizures observed in XXX-treated animals were less complex, typically initiated only during awake and active behavior, and consisted of rearing, falling and forelimb clonus (see attached video). Seizure duration in these drug-treated animals was significantly reduced (average 48.8 ±10.9 [sem] seconds; p <0.01, Student’s t test) (Figure 2). In addition, unlike vehicle-treated animals, XXX-treated animals immediately recovered from the ictal event and resumed normal behavior.
The total time animals spent in seizure activity was also significantly reduced in the XXX-treated group. Average time spent in ictal activity was 11.4 seconds/hour in the vehicle treated group. Administration of XXX reduced average ictal activity time to 3.25 seconds/hour.

In vehicle-treated animals, average Racine score was 4.3 ± 0.09 (sem). Administration of XXX reduced average Racine score to 3.6 ± 0.04 (sem), $p = .002$, Student’s t test) (Figure 3).

c. **Effect of XXX on the development of ictal activity over time.**

All animals were allowed to recover from SE for at least 3 weeks to allow for the development of recurrent seizures. In that time, all vehicle-treated animals developed recurrent seizures. Of XXX-treated animals, 3 out of 6 developed recurrent seizure activity. Once observed, ictal activity in both groups displayed a random occurrence, although one XXX-treated animal did display ictal activity that had a higher seizure frequency (Figure 4). Thus recurrent seizure activity in all animals studied was random and recurrent.

4. **Conclusion and Discussion**

This study was designed to determine the effect of XXX on recurrent seizure activity in the rat pilocarpine model, after the onset of status epilepticus (SE). XXX demonstrated protective effects as observed in the reduced probability of developing recurrent seizure activity, duration of seizure activity in animals that did develop recurrent seizure activity, and seizure severity as assessed by behavioral scores. The data demonstrated a substantial protective effect of XXX administration in this model.

Overall, XXX administration after the development of SE reduced the probability of developing recurrent seizures by fifty percent. In addition, one drug-treated animal that did display spontaneous seizure activity, displayed only a single ictal event. Thus, XXX reduced the development of recurrent seizure activity when compared to vehicle-treated controls. While other compounds have been shown to protect against the development of recurrent seizure activity in the pilocarpine model (e.g., MK-801 [13] and ketamine [15]), they either possess their own neuronal toxicity [16], or require administration prior to the induction of SE [13]. The protective effects of XXX were observed well after the development of drug-refractory SE. Thus, XXX is a unique compound in that it blocks recurrent seizure activity following establishment of SE in at least half the subjects. It is well known that SE is a major cause of symptomatic epilepsy [17-20], and that SE is the first seizure observed in multiple newly acquired epilepsies [19]. Therefore, the development of a compound that can be given after the onset of SE, and still prevent or reduce the severity of recurrent seizures, would be a significant addition to the treatment of this disease.
Another notable observation in this study was that administration of XXX reduced seizure severity in those drug-treated animals that did develop recurrent seizure activity. This was assessed by both behavioral (Racine) score as well as duration of ictal activity. SE is often a medical emergency, having both a high mortality rate (approximately 40%) [21] and inducing severe recurrent seizures in patients that do survive the SE event. Often, the recurrent seizure activity is refractory to current treatments, and requires polytherapy to keep under control. Should XXX reduce seizure severity in the patients who do develop recurrent seizures, it is possible that XXX will lessen the burden of victims of refractory SE. Reducing seizure severity, seizure frequency and therapeutic efficacy would be a significant advancement to current therapies available.

5. References

6. Figures

Figure 1. XXX reduced the probability of recurrent seizure activity. All vehicle-treated rats that developed SE also developed recurrent seizure activity whereas only half of XXX-treated animals developed seizure activity.

Figure 2. XXX significantly reduced seizure duration. Average seizure duration in vehicle-treated animals was 87.0 seconds. Administration of XXX reduced the average seizure duration by 44%, to 48.8 seconds. ** p < 0.01, Student's t test.
Figure 3. XXX significantly reduced seizure severity. Behavioral severity was assessed using the Racine Scale (Table 1). Average Racine score for vehicle treated animals was 4.3. Administration of XXX reduced seizure severity by 16%, to 3.6 ** p = .002, Student’s t test. Only animals that displayed seizures are included.

![Racine Score Chart](image)

Figure 4. Seizure frequency profile for all animals observed. Vehicle-treated animals (red symbols) displayed a random, non-clustering seizure frequency. Only 3 drug-treated animals (50%) displayed ictal activity during the 40 hour observational period.

Temporal Profile of Seizure Observations

![Seizure Frequency Chart](image)
APPENDICES

Raw data for this study is attached as a separate (Excel) file. Two video examples are also attached.