Canine status epilepticus treated with fosphenytoin: A proof of principle study

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SUMMARY

Objectives: There are a limited number of marketed intravenous antiepileptic drugs (AEDs) available to treat status epilepticus (SE). All were first developed for chronic therapy of epilepsy, not specifically for SE. Epilepsy and canine SE (CSE) occur naturally in dogs, with prevalence, presentation, and percentage of refractory cases similar to human epilepsy. The objective of this study was to determine if CSE treated with fosphenytoin (FOS) results in a similar responder rate as for people.

Methods: A randomized clinical trial was performed for dogs with CSE. Dogs who presented during a seizure or who had additional seizures after enrolling received intravenous (i.v.) benzodiazepine (BZD) followed immediately by intravenous infusion of 15 mg/kg phenytoin equivalent (PE) of fosphenytoin (FOS) or saline placebo (PBO). If seizures continued, additional AEDs were administered per the standard of care for veterinary patients. Total and unbound plasma phenytoin (PHT) concentrations were measured.

Results: Consent was obtained for 50 dogs with CSE. Thirty-one had additional motor seizures and were randomized to the study intervention (22 FOS and 9 PBO). There was a statistically significant difference in the 12 h responder rate, with 63% in the FOS group versus 22% in the placebo group (p = 0.043) having no further seizures. The unbound PHT concentrations at 30 and 60 min were within the therapeutic concentrations for people (1–2 l g/ml) with the exception of one dog. There was mild vomiting in 36% of the FOS group (7/22) within 20 min of FOS administration and none of the placebo group (0/9) (p = 0.064).

Significance: This proof of concept study provides the first evidence that FOS is tolerated and effective in canine SE at PHT concentrations clinically relevant for human SE. Furthermore, naturally occurring CSE can be utilized as a translational platform for future studies of novel SE compounds.

KEY WORDS: Translational, Dog, Seizure, Emergency, Animal model.

Human status epilepticus (HSE) is a serious life-threatening neurologic emergency consisting of prolonged and/or frequent seizures. HSE is associated with one of the highest mortalities and morbidities of any neurologic condition. It has been reported that 152,000 cases with 42,000 deaths (28%) occur each year in the United States.¹,² Treatment

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guidelines recommend use of benzodiazepines (BZDs) and phenytoin (PHT) or its prodrug fosphenytoin (FOS). These guidelines are based on studies initiated in the 1990s, with drugs developed 30 or more years ago for conditions other than the treatment of HSE. The most comprehensive study of HSE management was a comparison of four treatments: lorazepam; phenobarbital; diazepam followed by PHT; and PHT. PHT had a success rate of 43.6% when given alone, but the rate was 55.8% for diazepam followed by PHT. The best treatment, lorazepam, had a success rate of only 64.9%. There is a need to develop models that provide evidence that new drugs have potentially superior efficacy or safety compared with accepted therapy. Promising new agents discovered using experimental animal models of status epilepticus cannot easily be tested in HSE without more evidence of safety and efficacy. Related to this issue is a mechanism to develop and evaluate drugs that may be specifically effective for HSE and yet may, not be appropriate for chronic use. Treatment of HSE requires only short-term use of drugs in patients who have a decreased level of consciousness. However, using the conventional approach to development of new AEDs, significant adverse effects arising from chronic exposure, will preclude the development of these AEDs for SE. The ideal drugs for HSE are those that have a rapid onset of action, have neuroprotective activity, and are free from significant respiratory and cardiovascular effects.

In contrast to artificially induced seizures in rodent models, epilepsy occurs naturally and spontaneously in dogs. Rodent models also often fail to predict cognitive, behavioral, and neurologic side effects (irritability, insomnia, and balance) and systemic effects (cardiac arrhythmias and hypotension). The clinical manifestations of seizures in dogs are similar to those observed in humans, including seizure types and electroencephalographic findings. Naturally occurring canine status epilepticus (CSE) is one of the more common emergency conditions treated at veterinary hospitals. Approximately 59% of dogs with epilepsy have one or more episodes of CSE during their lifetime, and those with CSE had a mean life span of only 8.3 years compared to 11.3 years for those with epilepsy; but no CSE. Despite the similarities with epilepsy in humans, use of naturally occurring canine epilepsy for drug development has been underutilized. A positive proof-of-concept study with a drug that has been well documented to work in HSE would validate the model and could propel additional studies of novel drugs in CSE. Using dogs as a translational platform has the added advantage of more accurate dose extrapolation to people because body sizes of dogs are closer to humans than are those of rodents (rodent to human is 1:4). PHT can cause hypotension cardiac arrhythmias and serious infusion-site adverse effects. Fosphenytoin is a highly water-soluble prodrug of PHT, and circulating phosphatases convert FOS to PHT with a half-life of 8–15 min, and was chosen for this study due to less concern with cardiac, blood pressure, and infusion-site adverse effects. For novel drugs, once an effective blood level has been determined in dogs, a dose for humans to attain the same levels can be calculated using standard loading-dose equations, incorporating parameters such as the volume of distribution with the appropriate caution of species differences in metabolism, protein binding, and so on.

The goal of this study was proof-of-concept of using CSE as a translational platform with a drug, FOS, effective for HSE. Demonstrating similar efficacy with FOS would validate CSE as a translational platform for HSE and allow novel drugs to be moved more quickly from the laboratory into studies of HSE. In addition, compounds that may not be pursued for chronic treatment of human epilepsy can be evaluated in CSE as potentially suitable for HSE. As an additional benefit for veterinarians, the results of this study and future studies will also inform the treatment of CSE.

**Methods**

**Design**

This was a prospective, double-masked, randomized, placebo-controlled study. Each dog that conformed to the inclusion (established CSE) and exclusion criteria was enrolled. Institutional Animal Care and Use Committee (IACUC) or equivalent approvals were obtained at all sites.

**Inclusion criteria**

Dogs with a clinical diagnosis of convulsive status epilepticus defined as continuous convulsion lasting >5 min, or two or more recurrent convulsions without regaining consciousness between seizures.

**Study protocol**

Dogs presenting for the emergency treatment of seizures at four veterinary centers (University of Minnesota, University of Pennsylvania, Chicago Veterinary Neurology, and Bush Veterinary Neurology) were considered for entry. Informed consent was obtained directly from the owner of the dog. If the inclusion criteria were met, dogs were admitted to the veterinary hospital for monitoring for 5 h consisting of the following: (1) continuous observation for seizures; and (2) hourly checks for alertness, vomiting, diarrhea, or salivation, and measures of temperature, pulse, and respirations. Pretreatment complete blood count (CBC), serum chemistry, blood pressure, electrocardiography (ECG), and either bile acids or resting ammonia was obtained. If patients recovered fully, they were discharged from the study. If another seizure occurred during the 5 h monitoring or the dog presented during a seizure, 0.5 mg/kg of diazepam or 0.2 mg/kg lorazepam (when diazepam was not available) was given intravenously. The BZD dose was immediately followed by an intravenous loading dose of...
FOS of 15 mg/kg phenytoin equivalent (PE) at a rate of 50 mg PE/min (1 ml/min), or an equivalent volume of 0.9% NaCl at 1 ml/min was given. This dose of FOS in dogs was designed to attain plasma PHT levels similar to that when humans receive 18–20 mg/kg PHT.14 A rescue protocol (diazepam constant rate infusion (CRI), and/or i.v. levetiracetam, and/or i.v. propofol) per the veterinary standard of care15 was initiated if the dog’s convulsions did not diminish within 10 min after the completion of infusion or reoccurred within 12 h. Post study drug infusion monitoring consisted of blood pressure, 60 s ECG, and modified Glasgow Coma Scale score16 assessment at 10 and 20 min. In addition, vital signs were checked every hour for the entire 12 h.

**Primary end points**

The primary end points were an additional seizure within 2 and 12 h after completion of infusion. If there were no seizures within these time periods, the case was considered a responder.

**Secondary end points**

The secondary end points were number of seizures within 12 h, number of bolus injections of diazepam given, number of dogs receiving a constant rate infusion of diazepam and duration of infusion, number of dogs receiving either propofol treatment, hours of propofol CRI, and hours of hospitalization beyond 12 h.

**Plasma PHT concentrations**

Blood samples were collected at 0.5, 1.5, and 3 h after the end of the FOS infusion. A reversed phase, high-performance liquid chromatographic assay was used for measurement of bound and unbound PHT and was adapted for canine plasma using a previously validated method to measure bound and unbound PHT in human plasma.14 For unbound concentrations, 1 ml of plasma was spun using an Amicon Centrifree ultrafiltration device (Merck Millipore Ltd Billerica MA, U.S.A) at 37°C for 1 h at 1413 g to separate drug bound to proteins from unbound drug. For PHT measurement, sample extraction from canine plasma was accomplished with hexane and ethyl acetate followed by evaporation of the solvent under nitrogen in a 37°C water bath. Compounds were eluted using an ion-pair mobile phase containing 25% acetonitrile and 75% phosphate buffer (pH 3.5), and PHT was detected using UV absorbance at 215 nm.

The drug concentrations obtained from these subjects were used to confirm attainment of the target concentrations, and to examine the relationship between PHT concentration, seizure control, and adverse events.

**Power calculation and statistical analysis**

The primary end points were compared between groups using a Fisher’s exact test. Secondary end points were compared between groups using the Wilcoxon-rank sum test or a Fisher’s exact test. In addition, the age, sex, clinic location, and number of seizures in the 12 h prior to admission were compared between the two groups to determine if the groups are otherwise equivalent. Differences were considered statistically significant at a p < 0.05. Sample size and power estimates were based on a literature review of studies of three AEDs with human subjects as well as our experience with our canine LEV study.17 Sample size estimates with 80% power to detect a 40% difference between FOS (50% effective) and placebo (10%) for 12 h responders using a significance level (α) = 0.05 indicated a total sample size of 46 enrolled cases receiving study infusion. An a priori, planned interim analysis of 20 enrolled patients was performed to determine exact power calculations with this cohort of dogs with CSE. The initial 20 dogs were randomized to about one half FOS and one half placebo by a statistician using a random number generator. After the interim analysis, the remaining subjects were randomized at a ratio of 5 FOS to 1 PBO due to the potential futility of the placebo group with a 0% 12 h responder rate.

**RESULTS**

**Interim analysis**

This analysis showed that at 12 h, 69.2% (9/13) of the patients in Treatment Group A (later revealed as the FOS group) and 0% (0/7) of the patients in Group B (later revealed as PBO) were responders (p = 0.0031). At 2 h, Group A (FOS) had 100% (13/13) responders versus Group B (PBO) with 71% (5/7) responders (p = 0.11). With statistical significance already found for 12 h responders, it was calculated that a total of 32 subjects would be needed to potentially find a statistically significant difference at the 0.05 level for 100% versus 77% for the 2 h end point. Based on this power calculation, we decided to try enrollment of 12 additional dogs (for a total of 32) at a ratio of 5 Group A (FOS):1 PBO (Group B) in order to possibly achieve the power to detect a difference at 2 h, and also because of the apparent futility of Group B (PBO) in this population.

**Final study population**

Ultimately, consent was obtained from owners of 50 dogs with SE. One case presented during a seizure; 30 cases had additional motor seizures and were randomized to the study intervention at about an overall 2:1 ratio (22 in the FOS group and 9 in the PBO group). This occurred just as funding ended and was one case short of the goal of 32 total enrolled cases. Breeds with more than one enrolled case included five golden retrievers (three FOS, two PBO), four Labrador retrievers (one FOS, three PBO), three bulldogs (two FOS, one PBO), and three German shepherd dogs (two FOS, one PBO). There were 16 female dogs (12 FOS, 4
PBO), and 15 male dogs (10 FOS, 5 PBO). Six dogs in the FOS group had secondary epilepsy (one meningioma, one glioma, two inflammatory encephalitis, and two cases of hydrocephalus), and two dogs in the placebo group had inflammatory encephalitis. All other dogs in both groups (n = 23) met criteria for genetic (idiopathic) epilepsy.

There were no statistically significant differences between the FOS and PBO groups in pretreatment CBC, serum chemistry, bile acids, age, weight, sex, neuter status, underlying cause of seizures, number of doses of diazepam prior to study admission, or number of seizures in the 12 h prior to admission (Table 1).

Outcomes
There was a statistically significant difference in 12 h responder end point with 63.6% (14/22) in the FOS group versus 22.2% (2/9) in the placebo group (p = 0.043). There was also a significant difference for the 2 h responders end-point with 21/22 (95.4%) responders in the FOS group versus 5/9 (55.5%) in the PBO group (p = 0.017)—Table 2.

Pharmacokinetics
The PHT concentrations in plasma collected at 30, 60, and 120 min postdose (Fig. 1) were within the therapeutic concentrations for human subjects (7.5–20 µg/ml total and 1–2 µg/ml unbound) at 30 and 60 min, with the exception of one dog that had low total and unbound PHT concentrations at 30 min postdose of 7 and 0.7 µg/ml, respectively. There was no significant difference between total or unbound drug concentrations for responders versus nonresponders, although there was a potential trend toward greater unbound concentrations in responders at 30 min postdose (Fig. 2).

Adverse effects
There were no statistically significant differences for any adverse effects or other medical data variables (Table 2). Mild, drug-controllable vomiting was more common in the FOS group of 32% (7/22) versus 0% (0/9) in the PBO group, but this did not quite reach statistical significance in this number of patients (p = 0.065). The initial vomiting was within 20 min of FOS infusion completion in all seven dogs, with only one dog having a second episode at 40 min. Unbound PHT levels were similar at 30 min in the dogs that vomited (1.90 µg/ml) compared to the dogs that did not vomit (1.70 µg/ml) (p = 0.36). Dogs with vomiting were treated with maropitant or ondansetron, and no vomiting occurred beyond 40 min after completion of the study infusion. No aspiration pneumonia was documented to have occurred in any of the dogs. Due to the known risk of vomiting from FOS in dogs,15,18 it was recommended in the study

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**Table 1. Comparison of fosphenytoin (FOS)–treated and placebo-treated dogs with status epilepticus by age, weight, seizure frequency, etiology, and sex**

<table>
<thead>
<tr>
<th>End point/variable</th>
<th>FOS group (n = 22)</th>
<th>Placebo (n = 9)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (range)</td>
<td>6.5 (3–13)</td>
<td>4.4 (1.5–12)</td>
<td>0.10</td>
</tr>
<tr>
<td>Weight in pounds (range)</td>
<td>28.0 (10–58)</td>
<td>36.8 (29–48)</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean no. seizures within 12 h prior to admission (range)</td>
<td>6.4 (3–10)</td>
<td>5.6 (3–10)</td>
<td>0.59</td>
</tr>
<tr>
<td>Genetic (idiopathic epilepsy) (%)</td>
<td>16/22 (73)</td>
<td>7/9 (78)</td>
<td>0.57</td>
</tr>
<tr>
<td>Secondary epilepsy (%)</td>
<td>6/22 (27)</td>
<td>2/9 (22)</td>
<td>0.57</td>
</tr>
<tr>
<td>Male (%)</td>
<td>10/22 (45)</td>
<td>5/9 (55)</td>
<td>0.70</td>
</tr>
<tr>
<td>Female (%)</td>
<td>12/22 (55)</td>
<td>4/9 (44)</td>
<td>0.70</td>
</tr>
</tbody>
</table>

**Table 2. Representative primary and secondary end points and other important outcome variables comparing fosphenytoin (FOS) versus placebo in canine status epilepticus (CSE)**

<table>
<thead>
<tr>
<th>End point/variable</th>
<th>FOS group (n = 22)</th>
<th>Placebo (n = 9)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% responders at 2 h</td>
<td>21/22 (95)</td>
<td>5/9 (55)</td>
<td>0.017*</td>
</tr>
<tr>
<td>% responders at 12 h</td>
<td>14/22 (64)</td>
<td>2/9 (22)</td>
<td>0.043*</td>
</tr>
<tr>
<td>No. episodes of motor seizures &gt;5 min first 12 h (%)</td>
<td>1/22 (4.5)</td>
<td>0/9 (0)</td>
<td>0.71</td>
</tr>
<tr>
<td>Mean no. seizures 12 h post treatment (range)</td>
<td>0.61 (0–4)</td>
<td>1.54 (0–4)</td>
<td>0.020*</td>
</tr>
<tr>
<td>Vomiting (%)</td>
<td>7/22 (32)</td>
<td>0/9 (0)</td>
<td>0.065</td>
</tr>
<tr>
<td>In-hospital mortality (%)</td>
<td>3/22 (13)</td>
<td>1/9 (11)</td>
<td>0.63</td>
</tr>
<tr>
<td>Arrhythmia during or post infusion (%)</td>
<td>2/22 (9)</td>
<td>0/9 (0)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Statistically significant differences indicated with *.
protocol to keep the head lowered for 15 min post study drug infusion, to try to prevent aspiration. Occasional premature ventricular contractions occurred in two dogs in the FOS group (9%) just after the FOS infusion, but treatment was not indicated or initiated in either dog. Hypotension was not detected in any dogs, as systolic blood pressures were >80 mm in all dogs during and after the study drug infusion.

The overall in-hospital mortality was 12.9% (4/31), which is within the general expected range for dogs with SE.9–11 There was a 13.6% (3/22) mortality rate in the FOS group versus 11.1% (1/11) in the PBO group, which was not statistically significant difference (Table 2). The one death in the PBO group was an owner-elected euthanasia 16 h after study infusion in a dog that had stable vital signs but was a nonresponder with continuing seizures. In the FOS group, two of three deaths were also owner-elected euthanasia’s in stable nonresponders. One FOS case died just prior to 3 h post-FOS infusion. This dog did vomit one time, 1 min post-FOS infusion completion, and was treated with ondansetron, 0.2 mg/kg, i.v., at that time, and there was no additional vomiting. The dog had blood pressure, ECG, and respiratory rates within normal limits for the planned 10 and 20 min post infusion monitoring, and appeared stable until just prior to 3 h post-FOS infusion. At that time, the dog was found to be very weak and tachycardic, with pulse rate of 144 and a capillary refill time of >3 s. The dog was taken to the veterinary intensive care unit and shortly after went into respiratory arrest; cardiopulmonary resuscitation (CPR) was not performed due to a do not resuscitate status. The 30 and 60 min post infusion free PHT levels were 1.60 and 0.92 mg/dl, respectively, in this case, which was within the study target range. A necropsy (animal postmortem) was requested but was not approved by the owners.

**DISCUSSION**

The 64% responder rate for the 12 h end point in the BZD/FOS-treated dogs is similar to the 55.8% reported for people in the Trieman study.4 This study, therefore, met its aims by demonstrating that response to FOS of subjects with CSE is similar to that reported for HSE. The results of this study show that FOS is an effective drug for the treatment of CSE in dogs, with relatively few adverse events, except vomiting shortly after administration. At both 2 and 12 h there was a better responder rate for FOS versus PBO, and statistical significance can potentially be found with a limited number of CSE cases in a randomized-controlled clinical trial.

Study limitations include lack of electroencephalography (EEG) for nonconvulsive SE; not all cases were managed by the same clinician, and consequently there was some variation in treatments received (other than FOS), and magnetic resonance imaging (MRI) was not performed for all patients and therefore a final diagnosis was not precisely determined in all cases. Even with the lack of EEG, the results of CSE studies can be directly translatable to convulsive HSE, as evidenced by the similar response rate to FOS for SE in both species. In addition, it is not yet clear at what hour responder rate is the best translational model end point for CSE, although the 12 h end point for CSE does appear to be a reasonable clinical end point, as some of the dogs who were responders at 6 h, had additional seizures between 6 and 12 h.

Our results are significant because of the following: (1) the study offers proof-of-concept for future testing of the efficacy and safety of non–U.S. Food and Drug Administration (FDA) approved, investigational agents discovered in experimental models, which may be significantly superior in efficacy or neuroprotection to present treatments for humans; and (2) a new platform to speed the translation of experimental agents to human use has been developed. In this study, we established a national network of four veterinary emergency care departments to evaluate FOS in CSE. We have just expanded this network to seven veterinary centers and have future plans to test novel drugs in CSE that have shown promise in rodent SE models, in an attempt to initiate a process of efficient results for potential translation...
into HSE studies. If a drug has promising results in the canine platform, this could speed its development into human clinical trials, and if it is ineffective in canine SE, money could be saved in not pursuing development for HSE.

**Acknowledgments**

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**Disclosures**

None of the authors has any relevant conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

**References**