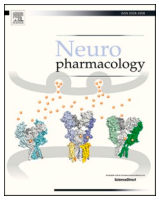
Neuropharmacology 222 (2023) 109310

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm



Soticlestat, a novel cholesterol 24-hydroxylase inhibitor, modifies acute seizure burden and chronic epilepsy-related behavioral deficits following Theiler's virus infection in mice

Melissa Barker-Haliski [a,*], Toshiya Nishi [b,c,**], H. Steve White [a]

[a] University of Washington, Department of Pharmacy, Seattle, WA, USA
[b] Research, Takeda Pharmaceutical Company Limited, Fujisawa, 251-8555, Japan
[c] Takeda Pharmaceutical Company Limited, Cambridge, MA, 02139, USA

ARTICLE INFO

Keywords:
Theiler's virus
Infection-induced epilepsy
Novel antiseizure drugs
Soticlestat
Cholesterol 24-hydroxylase

ABSTRACT

Temporal lobe epilepsy is the most common form of acquired epilepsy and can arise due to multiple inciting events, including central nervous system (CNS) infection. CNS infection with the Theiler's murine encephalomyelitis virus (TMEV) in male C57Bl/6J mice leads to acute, drug-resistant handling-induced seizures. Cholesterol 24-hydroxylase (CH24H) is a brain-specific enzyme that converts cholesterol into 24S-hydroxycholesterol; the primary mechanism of cholesterol catabolism in the brain. The novel CH24H inhibitor, soticlestat (SOT; or TAK-935), demonstrates the potential to restore excitatory/inhibitory balance in multiple preclinical models of hyperexcitability. This study thus sought to characterize the anticonvulsant potential of SOT in the TMEV model. Treatment with SOT (30 mg/kg, p.o.; n = 30) 0–7 days post-infection (DPI) reduced overall seizure burden and severity. SOT administration significantly delayed onset of infection-induced Racine stage 5 seizures, from 8.6 ± 0.6 (VEH-treated) to 10.8 ± 0.8 (SOT-treated) observation sessions. Infected mice were then allowed 36 days treatment-free recovery before assessing impact of earlier drug administration on epilepsy-related cognitive and behavioral comorbidities, including a non-habituated open field (OF) task. Total OF distance traveled was significantly less in SOT-treated mice compared to VEH-treated mice, suggesting attenuated TMEV-induced spatial memory deficits, or reduced chronic hyperexcitability. Mice with history of SOT treatment also spent significantly more time and traveled farther in the OF center, indicative of reduced epilepsy-induced anxiety-like behavior. These studies suggest that SOT is a mechanistically novel agent for symptomatic seizure control. Moreover, acute SOT administration during an epileptogenic insult may attenuate the resulting long-term behavioral comorbidities of epilepsy.

1. Introduction

Roughly 80% of the global burden of epilepsy is in low and middle-income countries (WHO, 2022) and central nervous system (CNS) infections are one of the main risk factors for epilepsy in resource-poor settings (Ba-Diop et al., 2014; Singh et al., 2008; Singh and Sander, 2020). CNS infection accounts for approximately 14.8% of newly diagnosed epilepsy in Ecuador (Carpio et al., 2001), whereas in the United States, CNS infection accounts for approximately 3% of new epilepsy cases (Annegers et al., 1995). Thus, infectious pathogens of the CNS are an underrecognized driver of the worldwide prevalence of epilepsy.

In terms of specific pathogens, infection with human herpes 6B virus is associated with the development of encephalitis, seizures, and epilepsy (Misra et al., 2008). Neurocysticercosis is the most common helminthic infection of the CNS and frequently leads to acute seizures and epilepsy, particularly in susceptible regions of the world (Del Brutto, 2016). Even SARS CoV-2 infection has been documented to induce rare instances of acute encephalopathy and acute seizures (Galanopoulou et al., 2020), which may further increase the global incidence of viral infection-induced epilepsy. Treatments that can control acute seizures resulting from CNS infections may reduce subsequent development of epilepsy and thereby be a strategy to meaningfully reduce the global burden of this disease.

Viral infections of the CNS can contribute to the development of

* Corresponding author.
** Corresponding author. Research, Takeda Pharmaceutical Company Limited, Fujisawa, 251-8555, Japan.
E-mail addresses: mhaliski@uw.edu (M. Barker-Haliski), toshiya.nishi@takeda.com (T. Nishi).

https://doi.org/10.1016/j.neuropharm.2022.109310
Received 27 July 2022; Received in revised form 17 October 2022; Accepted 24 October 2022
Available online 29 October 2022

epilepsy due to an increased risk of seizures and status epilepticus (Barker-Haliski et al., 2017). Patients with viral infection-induced encephalitis who present with seizures during the acute infection period are up to 22 times more likely to develop spontaneous, unprovoked seizures than the general population (Misra et al., 2008). Infection-induced CNS inflammation represents a significant risk factor for seizure induction and maintenance largely due to the surge in inflammatory cytokines observed in animal seizure models and patients with epilepsy.

CNS infection of C57BL/6J mice with Theiler's murine encephalomyelitis virus (TMEV) is a valid preclinical model of infection-induced epilepsy that is well-suited to elucidate mechanisms of epileptogenesis following a brain infection (Broer et al., 2016; Sanchez et al., 2019; Waltl et al., 2018) and identify novel pharmacotherapies for the treatment of seizures or prevention of epilepsy (Barker-Haliski et al., 2015, 2016a; Libbey et al., 2016b; Loewen et al., 2019; Patel et al., 2017, 2019). A large majority of TMEV-infected animals develop acute, handling-induced seizures after the initial infection and show significant elevations in inflammatory cytokines (Libbey et al., 2008). Roughly 50% of the mice that initially develop handling-induced seizures go on to subsequently develop spontaneous, recurrent seizures weeks later (Stewart et al., 2010a, 2010b). Some agents are effective against acute seizures (Barker-Haliski et al., 2015; Metcalf et al., 2022; Patel et al., 2019; Waltl et al., 2018) and anti-inflammatory, but not anticonvulsant agents, may be particularly effective in their ability to modulate the development of chronic behavioral comorbidities that arise weeks after viral infection (Barker-Haliski et al., 2016a). Given the biphasic characteristic of both an acute period of evoked seizures and later epileptogenesis with spontaneous recurrent seizures (SRS) and behavioral comorbidities, the TMEV model is uniquely positioned to identify both mechanistically novel antiseizure agents, as well as potential therapies for epilepsy.

Soticlestat (SOT) is an investigational drug in clinical development for the treatment of developmental epileptic encephalopathies (DEE), such as Dravet syndrome and Lennox Gastaut syndrome. SOT was identified as a small-molecule inhibitor of CYP46A1 (also known as cholesterol 24-hydroxylase or CH24H). It potently inhibits CH24H at the IC50 of 4.5 nM (Nishi et al., 2020) while ensuring a high degree of selectivity against drug metabolizing CYP enzymes (Koike et al., 2021). Cholesterol 24-hydroxylation is known as a CNS-specific mechanism to oxidize cholesterol. Interestingly, its metabolite, 24S-hydroxycholesterol has been implicated in a wide range of biological functions that could underlie various epilepsies, including glutamatergic overactivity and inflammation (Alexandrov et al., 2005; Paul et al., 2013). With a high specificity to CH24H and brain penetration, SOT has been investigated as a chemical tool with a novel mechanism of action for the symptomatic treatment of epilepsy (Nishi et al., 2020). It possesses anticonvulsant efficacy in several well-characterized rodent seizure and epilepsy models (Nishi et al., 2022). Further, SOT demonstrated statistically significant, clinically meaningful reductions from baseline in median seizure frequency in patients with Dravet syndrome and Lennox Gastaut syndrome (Hahn et al., 2022).

The goal of this study was thus to determine the effects of an 8-day SOT treatment regimen on handling-induced seizures in the TMEV model. Mice were then allowed to recover for 36 days post-infection (DPI) and challenged in behavioral tasks representative of epilepsy comorbidities. This present study demonstrates that SOT administration was able to reduce handling-induced seizures during the acute phase of TMEV infection in mice and modify performance in the OF test weeks later. This study supports further evaluation of SOT in other chronic epilepsy models associated with inflammatory processes and points to of CH24H inhibition as a viable treatment option for epilepsy.

2. Methods and materials

2.1. Animal handling and drug dosing

Male, C57BL/6J mice (4–5 weeks; Jackson Labs, Bar Harbor, ME) were divided into two treatment groups (total n = 60). Group 1 (n = 30) received chronic SOT (provided by Takeda Pharmaceuticals, Fujisawa, Japan) in 0.5% MC vehicle once per day (30 mg/kg, p.o.) for 0–7 DPI. Group 2 (n = 30) received an equivalent volume of chronic MC vehicle (p.o.) once per day for Day 0–7 of the acute study. The treatment regimen of SOT followed the previously described treatment approach (Nishi et al., 2022). Animals were infected with TMEV 1 h after drug dosing on Day 0, as described below. Body weights were recorded daily during the entire study duration (8 days). Mice were group-housed (10 animals/cage) for the duration of the sub-chronic dosing and acute behavioral testing. Following the acute infection period, mice were challenged on the fixed speed rotarod on the 12th DPI to assess any potential for treatment-related latent motor impairment or delays in recovery from the viral infection, as previously reported (Barker-Haliski et al., 2015). Animals had access to food and water *ad libitum*. All animal experimentation was approved by the University of Utah Institutional Animal Care and Use Committee (protocol #12–04011) and conformed to ARRIVE guidelines (Kilkenny et al., 2010).

2.2. Surgical preparation and TMEV injections

One hour following drug dosing on Day 0, mice were anesthetized with 3% isoflurane, with anesthesia confirmed by lack of response to strong tail pinch. Mice were then injected intracerebrally (i.c.) with 20 μL TMEV (titer concentration of 2.5×10^5 plaque forming units (PFU)), as described previously (Barker-Haliski et al., 2015, 2016a; Libbey et al., 2008). All surgical sites were disinfected with 70% isopropyl alcohol prior to i.c. Injections. All surgical procedures were performed under sterile conditions. Following surgical procedures, animals were monitored until they had recovered from anesthesia. All animal care and use was approved by the University of Utah Institutional Animal Care and Use Committee.

2.3. Assessment of handling-induced seizures

Following TMEV infection, animals were assessed twice daily for seizure manifestation and body weights recorded daily for 0–7 DPI according to methods previously described (Barker-Haliski et al., 2015, 2016a; Stewart et al., 2010a). All seizure scoring was performed by investigators blinded to treatment group. In addition to measuring seizure severity, a general assessment of disease severity was determined by monitoring motor function. Animals received SOT (30 mg/kg, p.o.) or VEH each morning, then 1 h later were subjected to behavioral assessment consistent with our drug screening protocol in this model (Barker-Haliski et al., 2015, 2016a). Each morning and afternoon 2–7 DPI, animals were tested for handling-induced seizures and motor function (6 h separation between morning and afternoon testing sessions; 7 h separation between morning drug administration and afternoon seizure testing). Motor function was assessed with a righting reflex test, which was scored as follows: score 0, healthy mouse resists being turned over by the twisting of the proximal end of the tail to the right and left; score 1, mouse immediately rights itself on one side; score 1.5, mouse immediately rights itself on both sides; score 2, mouse rights itself in 1–5 s; score 3, righting takes more than 5 s; score 4, mouse unable to right itself. The presence and severity of handling-induced seizures and seizure severity was determined according to the Racine rating scale, with minor modification as follows: 0.5 - freezing behavior >5 s; 1 - freezing, jaw chomping and facial clonus; 2 - head bobbing; 3 - forelimb clonus; 4 - rearing with forelimb clonus; 5 - rearing and falling.

2.4. Open field (OF) activity assessment

All surviving mice were allowed to recover to 36 DPI for subsequent evaluation of potential behavioral deficits in an OF, as described previously (Barker-Haliski et al., 2016a; Umpierre et al., 2014). Briefly, a mouse was placed into an OF Plexiglas chamber (40L × 40W × 30H cm) equipped with infrared sensors to detect animal movement for 30 min. During the 30-min period, the total distance traveled (cm) and vertical activity counts were measured and recorded by an automated computer system (Accuscan, Omnitech, Inc). The measurements were specifically recorded in the OF center and perimeter, with total time spent in the OF center recorded as an additional measure of the anxiety-like behavioral comorbidity of epilepsy. Mice were analyzed both for all infected subjects, as well as within groups with and without acute seizures 0–7 DPI.

2.5. Novel object-place recognition (NOPR) task of object recognition memory

Following evaluation of behavioral performance in the OF, a subset of mice (n = 20/treatment group) were then challenged to perform in the novel object-place recognition task at 45 DPI, a measure of object recognition memory, as previously described (Barker-Haliski et al., 2016b). The task tests the mouse's ability to recall a novel object from a familiar object (Antunes and Biala, 2012; Hammond et al., 2004). In this task, learning is assessed by first exposing a mouse to a pair of identical "familiar" objects for 15 min at an approximate distance of 15 cm apart. After a 5-min retention interval where the mouse is isolated from the test environment, one "familiar" object is replaced with a "novel" object in a new location and the mouse's behavior is recorded for an additional 5 min. The time that the mouse spends with the novel object versus the time that is spent with the familiar object is determined based on the total time spent with both objects, representing a "recognition index" or RID score. Mice were analyzed both for all infected subjects, as well as within groups with and without acute seizures 0–7 DPI.

2.6. Statistical analysis

Daily body weights were analyzed by repeat measures ANOVA and post-hoc Sidak tests. Latency to first seizure Kaplan-Meier curves were determined with a Log-rank (Mantel-Cox) test. Proportion of mice with seizures (all and generalized Racine stage 3–5) was analyzed by Fisher's exact test. Effect of treatment on seizure burden and average number of generalized stage 3–5 seizures was evaluated by Kruskal-Wallis test. Long-term studies of behavioral comorbidities (rotarod, OF, NOPR) were analyzed by unpaired t-tests. All statistical analyses were conducted with GraphPad Prism v.6.0 or later, with $p < 0.05$ considered significant.

3. Results

3.1. SOT administration for 0–7 DPI improves TMEV-induced body weight loss

Body weight was recorded daily through 12 DPI of the study period (Fig. 1). Acute TMEV infection generally reduced body weight 0–7 DPI (Fig. 1A; ($F(9, 513) = 5.297$, $p < 0.0001$). *Post-hoc* tests revealed no significant differences between groups on any experimental day. Administration of SOT for 0–7 DPI significantly attenuated the TMEV-induced change in body weight from 0 DPI levels relative to VEH-treated, TMEV-infected controls. There was a significant time × treatment interaction, with SOT-treated mice showing less severe TMEV-induced weight loss over the course of the 8-day observation period (Fig. 1B; repeat measures ANOVA ($F(9, 513) = 5.244$, $p < 0.0001$). Post-hoc analysis for each experimental day demonstrated that the overall percent change in body weight in SOT-treated mice was significantly different from VEH-treated mice on Day 12 post-TMEV infection (Fig. 1B; $p < 0.01$). However, treatment with SOT significantly reduced the extent of TMEV-induced body weight loss versus VEH-treatment alone (Fig. 1). All animals in each treatment group demonstrated normal righting reflex. Finally, by 12 DPI animals in each experimental group were no longer experiencing behavioral seizures such that rotarod performance could be recorded, demonstrating no gross differences in motor impairment or recovery rate following the viral infection, as demonstrated by no detectable coordination deficits between the treatment groups (Fig. 1C; $t = 0.9012$, $p > 0.05$). These data cumulatively suggest that SOT treatment in TMEV-infected mice generally reduced infection-related body weight loss relative to VEH-treated, TMEV-infected mice and did not negatively affect post-infection recovery.

3.2. SOT reduces the proportion of mice presenting with acute racine seizures and reduces the maximum observed seizure severity during acute TMEV infection

TMEV-infection induces both acute behavioral seizures (Stewart et al., 2010a, 2010b), and also movement arrest and freezing for a duration of greater than 5 s with hunched, stereotyped posture (Barker-Haliski et al., 2015, 2016a). There were 24 VEH-treated mice (80%) and 19 SOT-treated mice (63.3%) that presented with movement arrest and/or seizure of any severity; this difference between treatment groups did not achieve statistical significance (Fig. 2A). However, there was a significant difference in the proportion of mice with and without generalized Racine stage 3–5 seizures (Fig. 2B; $p = 0.0089$). SOT-treatment from 0 to 7 DPI reduced the proportion of mice with stage 3–5 seizures by 50%: 22 VEH-treated mice (73.3%) with seizure, whereas only 11 SOT-treated mice (36.7%) presented with stage 3–5 seizure. Thus, acute SOT administration markedly reduced the severity of seizures, but did not prevent overall presentation of infection-induced

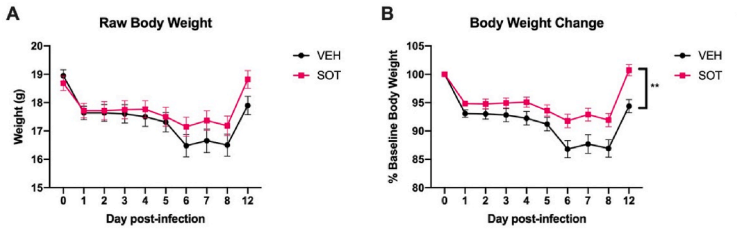


Fig. 1. Effect of 8-day administration of VEH (0.5% MC) or SOT (30 mg/kg, i.p., b.i.d.) on TMEV-induced changes in A) raw body weight and B) overall change in male C57Bl/6J mice. A) TMEV infection generally induced a significant decrease in body weight relative to Day 0 wt, and there was a significant treatment x time post-infection interaction on total body weight by 12 DPI ($F(9, 513) = 5.297$, $p < 0.0001$). However, there were no post-hoc Sidak's test differences between groups within each experimental day. B) Administration of SOT for 0–7 DPI significantly attenuated the TMEV-induced change in body weight from 0 DPI levels relative to VEH-treated, TMEV-infected controls. There is a significant time × treatment interaction, with SOT-treated mice losing less weight over the course of the 8-day observation period than VEH-treated mice (two-way ANOVA ($F(9, 513) = 5.244$, $p < 0.0001$). Post-hoc Sidak's analysis for each study day demonstrated that the overall percent change in body weight in TAK-treated mice was significantly different from VEH-treated mice on Day 12 post-TMEV infection (**$p < 0.01$).

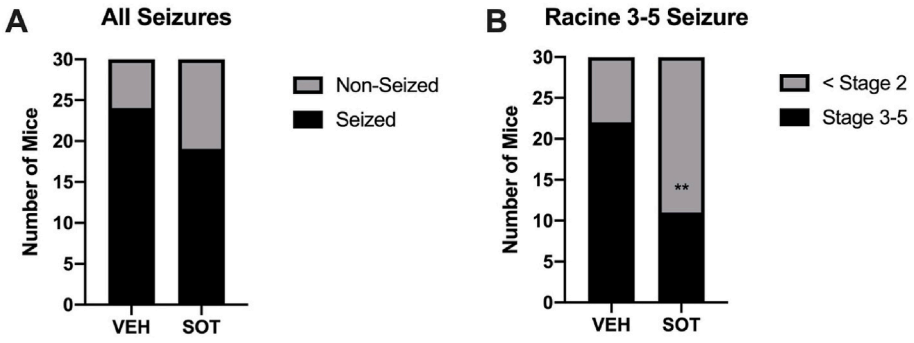


Fig. 2. Proportion of mice with and without seizures following TMEV infection and proportional distribution of maximum observed seizure severity in both treatment groups across all observation sessions 0–7 DPI. A) TMEV-infected mice presented with handling-induced seizures regardless of treatment with either VEH or SOT from 0 to 7 DPI. There was no statistical difference between the proportion of mice with seizures of any Racine stage (focal or generalized) or movement arrest (e.g., freezing >5 s) in either treatment groups ($p = 0.076$), as assessed by Fisher's exact test. B) However, when the proportion of mice with generalized Racine stage 3–5 seizures was similarly assessed, treatment with SOT significantly reduced the total number of mice that presented with a generalized Racine stage 3–5 seizure 0–7 DPI (*$p = 0.089$).

acute seizures.

Seizure burden is a means to qualitatively demonstrate overall changes in disease burden across time in chronic seizure and epilepsy models (Barker-Haliski et al., 2016a, 2021; Patel et al., 2017). This outcome measure is defined as the sum of the total seizure score per treatment group, with cumulative seizure burden representing the progressive increase in burden across the study period. SOT administration from 0 to 7 DPI reduced the overall seizure burden during the acute TMEV infection (Fig. 3A). The average seizure burden (Fig. 3B), average number of handling-induced seizures (Fig. 3C), and average number of generalized stage 3–5 seizures (Fig. 3D) were also compared between each treatment group across all handling-induced seizure observation periods following TMEV infection. Treatment with SOT significantly reduced the average seizure burden (Fig. 3B; $U = 269.5$, $p = 0.0065$). Similarly, the number of seizures was significantly reduced with SOT treatment (Fig. 3C; $t = 2.15$, $p = 0.036$). Finally, the average number of generalized stage 3–5 seizures was significantly different between treatment groups (Fig. 3C; $t = 2.608$, $p = 0.012$). Thus, administration of SOT 0–7 DPI meaningfully reduced several metrics of acute disease burden following CNS infection with TMEV in C57BL/6J mice, supporting the profile of acute anticonvulsant efficacy of this compound.

3.3. SOT delays the latency to onset of stage 5 seizures

TMEV infection typically results in acute, handling-induced behavioral seizures in mice by 3–5 DPI (Libbey et al., 2008). In this study, SOT administration significantly delayed the latency to onset of seizures of any Racine stage severity (i.e., stages 1–5; Fig. 4A; $X^2 = 6.072$, $p = 0.014$). Additionally, repeated SOT administration significantly delayed the presentation of Racine Stage 5 seizures (Fig. 4B; $X^2 = 6.072$, $p = 0.031$). Significantly more observation sessions elapsed before mice presented with a Stage 5 seizure (not shown; $t = 2.09$, $p = 0.041$).

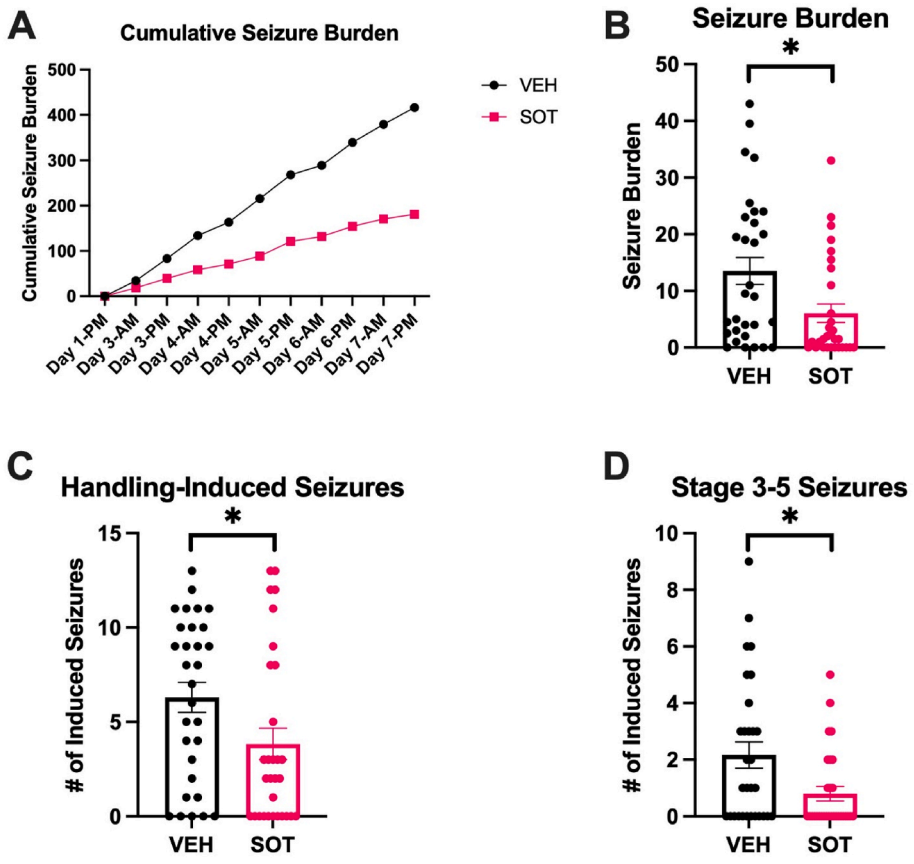


Fig. 3. For all mice in each treatment group enrolled in the study, the average seizure burden, number of handling-induced seizures, and average number of handling-induced generalized stage 3–5 seizures were compared between VEH- and TAK-treated mice. Subchronic treatment with SOT (30 mg/kg, p.o.) resulted in a significant reduction in all endpoints. A) There was a significant difference between treatment groups in the average seizure burden, $p = 0.0065$, as assessed by Mann-Whitney *U* test. B) There was a significant difference between treatment groups in the average number of handling-induced seizures, $p = 0.037$, as assessed by Student's *t*-test. C) There was a significant difference between treatment groups in the average number of handling-induced generalized stage 3–5 seizures, $p = 0.012$, as assessed by Student's *t*-test (D).

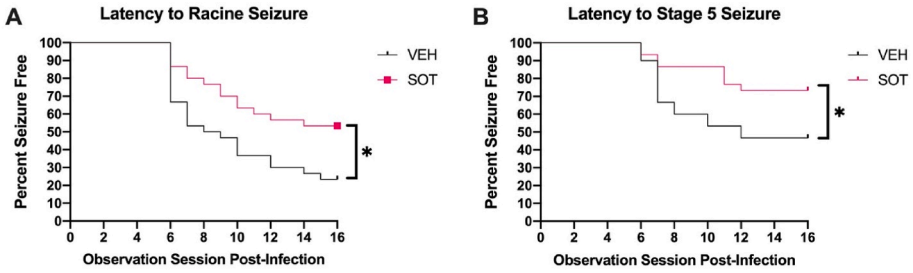


Fig. 4. Treatment with SOT significantly increased the latency to first Racine stage seizure of any severity and the latency to first generalized Racine stage 5 seizure. A) Latency to first Racine stage seizure is significantly improved with SOT treatment during the acute seizure phase (*p = 0.014). Latency to first Racine stage seizure was analyzed with a Log-rank (Mantel-Cox) test. B) Treatment with SOT significantly delayed the presentation of the first generalized Racine stage 5 seizure (*p = 0.031).

Altogether, these findings indicate that repeated SOT administration at 0–7 DPI delays onset of evoked Racine seizures, including generalized, stage 5 seizures, after intracerebral TMEV infection.

3.4. SOT administration during active TMEV infection blunts later severity of epilepsy-related anxiety-like behaviors in an open field (OF)

Animals treated with SOT or VEH during the acute infection period were allowed to recover to at least 36 DPI with no additional treatment before being challenged in the non-habituated OF task and NOPR task; two behavioral paradigms known to be negatively impacted by TMEV-infection at this time point (Barker-Haliski et al., 2015, 2016a; Umpierre et al., 2014). However, one mouse in the VEH-treated group died prior to behavioral testing (11 DPI), thus all OF studies were conducted with n = 30 SOT-treated mice and n = 29 VEH-treated mice. Mice acutely treated with SOT from 0 to 7 DPI traveled significantly less total distance in the OF at 36 DPI relative to VEH-treated mice (Fig. 5A; $t = 2.08$, $p = 0.042$). However, the proportion of the total exploration time elapsed within the OF center was significantly greater in SOT-treated mice relative to VEH-treated mice (Fig. 5B; $t = 2.29$, $p = 0.026$). Additionally, SOT-treated mice spent a significantly greater amount of their total exploration distance within the OF center (Fig. 5C; $t = 2.51$, $p = 0.015$). Total vertical rearing activity of VEH and SOT-treated mice did not differ (Fig. 5D; $t = 0.75$, $p > 0.4$), nor was there any difference in OF center vertical rearing activity (Fig. 5E; $t = 1.4$, $p = 0.17$). Therefore, twice-daily treatment of mice with SOT from 0 to 7 DPI led to significant reductions in thigmotaxis of mice weeks after the viral infection period, as measured by increased percent of total exploration time and duration within the OF center, indicative of a notable effect on anxiety-like behaviors, a comorbidity of chronic epilepsy in this animal model, and in humans with epilepsy.

3.5. Acute administration of SOT during the TMEV infection did not improve object recognition memory in the novel object-place recognition (NOPR) task by 45 days post-infection

Following a recovery period from the acute viral infection and SOT administration, TMEV-infected mice were challenged to perform in the NOPR task of object recognition memory at 45 DPI. This time point has been previously shown to coincide with SRS onset (Stewart et al., 2010a) and cognitive deficits (Umpierre et al., 2014). There was no statistical difference in the performance of either treatment group in the RID ($t = 0.65$, $p > 0.5$; Fig. 6A). When only the mice that experienced acute, handling-induced seizures during the active infection period were included in analysis, there was also no significant difference between groups ($t = 0.5059$, $p > 0.05$; Fig. 6B), highlighting that acute SOT administration did not lead to measurable improvements or deficits in performance relative to VEH-treated, TMEV-infected mice. The mice in both treatment groups demonstrated a preference for the novel object relative to the familiar object (VEH-treated: $t = 3.12$, $p = 0.004$; SOT-treated: $t = 2.86$, $p = 0.007$; data not shown). Furthermore, the percent of total time spent exploring the novel object did not differ between groups ($t = 0.67$, $p > 0.5$; Fig. 6C). When only the mice that experienced acute, handling-induced seizures during the active infection period were included in analysis of total time spent exploring the novel object, there was also no difference ($t = 0.8416$, $p > 0.05$; Fig. 6D). Thus, despite improvements in acute behavioral seizure incidence and chronic OF exploration for SOT-treated mice, object recognition memory performance in the NOPR task at 45 DPI did not differ between SOT and VEH-treated animals.

4. Discussion

The acute, handling-induced seizure phase of TMEV infection in

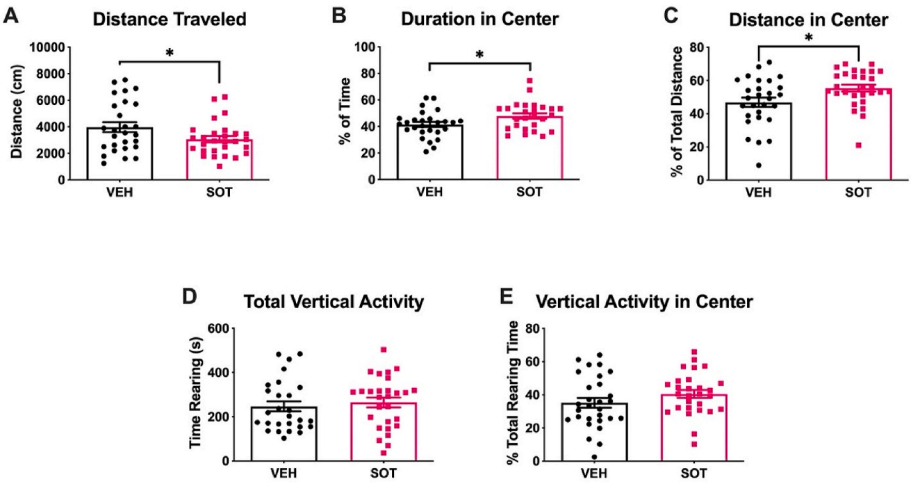


Fig. 5. The total distance traveled in an open field was evaluated for TMEV-infected mice 36 DPI and was analyzed by Student's t-test. A) Relative to VEH-treated mice, SOT-treated mice traveled significantly less total distance in the open field during the evaluation period. B) However, SOT-treated mice spent a significantly greater percent of their total exploration time in the center of the open field. * indicates significantly different from VEH-treated, TMEV-infected mice (Student's t-test), p = 0.026. C) SOT-treated mice spent a significantly greater percent of their total exploration distance in the center of the open field. * indicates significantly different from VEH-treated, TMEV-infected mice (Student's t-test), p = 0.015. D) The total vertical activity in an open field was evaluated for TMEV-infected mice 36 DPI and was analyzed by Student's t-test. However, there was no statistical difference in vertical rearing activity between VEH- and SOT-treated mice in the open field during the evaluation period. E) There was also no statistical difference in the proportion of total rearing time in the center zone of the open field.

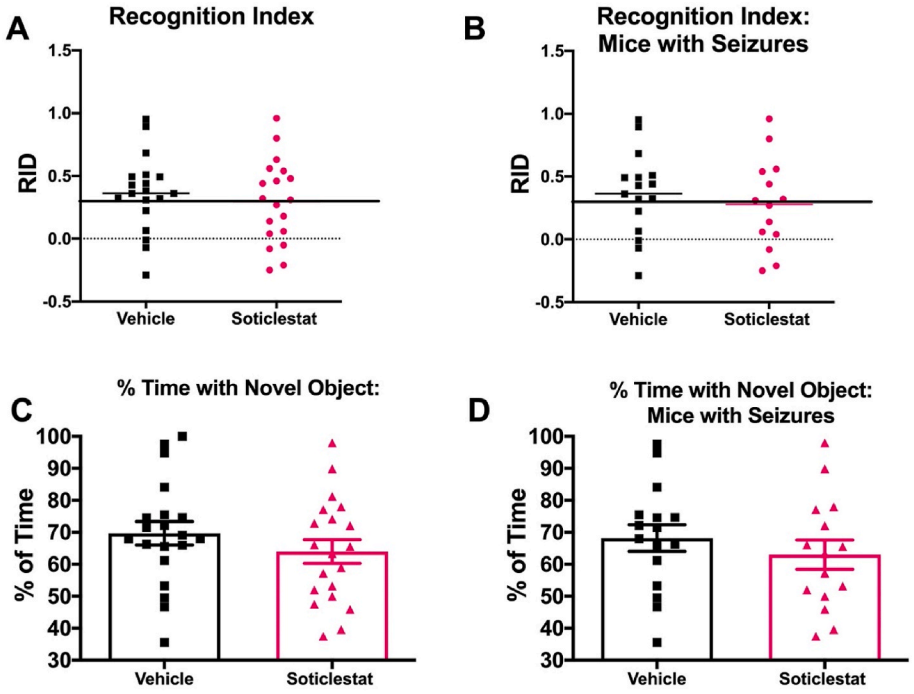


Fig. 6. Acute administration of SOT during acute TMEV infection did not markedly affect the preference for a novel versus a familiar object of mice 45 DPI. A) The recognition index (RID) score is a measure of preference for a novel object, and is defined by ((time novel − time familiar)/(time novel + time familiar). RID values greater than 0.25 indicate positive performance in this task (black line), indicating that no treatment group demonstrated significant impairment in performance. Furthermore, there was no statistical difference between treatment groups in the RID scores ($p > 0.5$). B) When only mice with acute seizures during the active infection were similarly analyzed, there was no significant difference between treatment groups. C) There was also no statistical difference between treatment groups in the percent of total exploration time that was spent with the novel object ($p > 0.5$). D) When only mice with acute seizures during the active infection were similarly analyzed, there was no significant difference between treatment groups.

male C57Bl/6J mice is useful to assess the antiseizure potential of investigational compounds and probe effects of treatment intervention on the epileptogenesis process itself (Barker-Haliski et al., 2016a). In this study, SOT was administered once daily during the acute TMEV infection period 0–7 DPI. Treatment was then stopped until mice were assessed between 36 and 45 DPI for performance in behavioral assays of epilepsy comorbidities, including anxiety-like behaviors, spatial working memory, and object recognition memory. In this approach, we not only assessed the acute anticonvulsant potential of SOT in a unique model of evoked seizures, but subsequently evaluated the effects of SOT on the comorbidities associated with mouse model of infection-induced epileptogenesis. SOT administration during acute infection effectively reduced the overall severity and incidence of seizures in this mouse model of viral infection-induced encephalitis. Treatment with SOT during the acute viral infection period (0–7 DPI) significantly blunted TMEV-induced body weight loss relative to VEH-treated, TMEV-infected mice. SOT significantly increased the number of days that elapsed until the first observed handling-induced Stage 5 seizure. SOT administration also significantly reduced the number of Stage 5 seizures observed during each twice-daily handling session. Finally, a significantly reduced number of mice experienced at least one observed Stage 5 seizure in the SOT-treatment group (8/30; 26.7%) versus the VEH-treatment group (16/30; 53.3%), indicating that acute SOT treatment during the TMEV infection period can markedly attenuate encephalitis-induced seizure susceptibility and overall seizure burden. SOT represents a potentially efficacious investigational compound for the attenuation of acute viral infection-induced seizures. More broadly, however, this study demonstrates that SOT possesses anticonvulsant potential in a mouse model of seizures characterized by neuroinflammatory pathogenesis.

Upon completion of the acute behavioral seizure monitoring period (0–7 DPI), mice were allowed to recover to at least 36 DPI for assessment of activity and object recognition memory in the NOPR task. It is important to note that the 30-day withdrawal period was long enough for elimination of SOT exposure and reversal of its direct pharmacodynamic effects (Nishi et al., 2020). Considering the 30-day washout period included in this present experiment, it is unlikely that the observed OF and NOPR behavioral effects are attributed to sustained reduction of 24HC because we previously confirmed a 24HC lowering approximately by 50% returns to the baseline level in 3 days after cessation of SOT administration. Clinical observations in the soticlestat phase1a/2b clinical trial confirm these earlier findings, wherein plasma 24HC levels were that were initially reduced ~80% during treatment recovered to pretreatment levels within a month after the treatment period (Halford et al., 2021). Results of our chronic behavioral comorbidities studies may suggest potential effects on neuropathogenic processes themselves because of short-term SOT administration relative to VEH-treated, TMEV-infected mice. Specifically, SOT-treated, TMEV-infected mice traveled less total distance in an OF, suggesting that the SOT treatment from Days 0–8 in the acute infection phase reduced the later development of chronic anxiety-like manifestations; a behavioral comorbidity of chronic epilepsy in the TMEV model (Umpierre et al., 2014). OF exploration is also a useful test to measure spatial memory in laboratory rodents; animals with hippocampal lesions and spatial memory deficits tend to ambulate to a greater extent in an OF (Praag et al., 1994; Walker et al., 2011). While our study was limited to only the assessment of TMEV-infected mice without a sham-infected control cohort, our study does demonstrate that SOT-treated mice spent more total exploration time in the OF center zone and traveled more distance within the OF center zone. Despite these observations, there was no significant difference between groups in the NOPR task 45 DPI, a measure of short-term (e.g., 5-min delay) object recognition memory. TMEV infection often induces chronic deficits in object recognition memory in drug-naive animals (Umpierre et al., 2014) and NOPR performance of similarly aged naïve male C57Bl/6 mice is sensitive to acute scopolamine administration (Barker-Haliski et al., 2016b), but in the absence of comparative evaluations with sham-infected mice in this present study, it is currently unclear whether the presently TMEV-infected mice had long-term memory deficits. The NOPR is a non-aversive, innate model of recognition memory (Cohen and Stackman, 2015) that challenges the ability of rodents to discern a novel object from a previously presented, familiar object (Antunes and Biala, 2012). This behavioral task is well-suited to drug discovery (Barker-Haliski et al., 2016b; Grayson et al., 2015) and relies predominately on functioning of the perirhinal cortex (Hammond et al., 2004), rather than the dorsal hippocampus that is known to be severely damaged after acute TMEV

infection (Barker-Haliski et al., 2015; Umpierre et al., 2014). Future studies are thus necessary to determine any effects on cognitive function resulting from SOT treatment during active TMEV infection. SOT administration during the acute seizure period nonetheless beneficially modified measures of chronic behavioral comorbidities that are otherwise known to be worsened by intracerebral TMEV infection (Barker-Haliski et al., 2016a; Umpierre et al., 2014).

SOT has recently emerged as a potential antiseizure medication with a novel mechanism of action (Nishi et al., 2020). Although the role of CH24H in epilepsy has not attracted a widespread attention as a promising therapeutic target, the antiseizure potential of SOT has been established in multiple animal models (Hawkins et al., 2021; Nishi et al., 2022). Among the known functions of 24S-hydroxycholesterol, allosteric modulation of NMDA receptors may deserve attention in this study. In theory, pharmacological reduction of 24S-hydroxycholesterol can result in a modification of NMDA receptor function, however, the antiseizure profile of SOT is hardly reminiscent of NMDA receptor blockers (Barton et al., 2003). In fact, MK-801 was previously found to be ineffective in TMEV model (Libbey et al., 2016b). Of note, CH24H inhibition does not cause worsening of recovery from acute viral infection, as demonstrated by performance on a rotarod 12 DPI (Fig. 1C). It is thus suggested that CH24H inhibition does not indiscriminately dampen baseline neural activities but rather, may protect the brain from pathologically induced excitatory/inhibitory imbalance. This interpretation agrees with the previous finding that SOT was ineffective in traditional models where seizures are acutely evoked in neurologically intact animals, such as maximum electroshock seizure and 6 Hz psychomotor seizure (Nishi et al., 2022). One interesting hypothesis arising from the present study is that CH24H inhibition exerts a therapeutic effect specifically in brains characterized by an altered inflammatory milieu. For example, innate immune responses have been implicated in the TMEV model (Kirkman et al., 2010). Future studies aimed at defining the involvement of 24S-hydroxycholesterol in inflammatory conditions could be relevant to treating neurological diseases, including epilepsy. It thus seems fair to infer that the effects of SOT observed in this study are mediated through a mechanism distinct from that of other known antiseizure medications.

5. Conclusions

This study further demonstrates that the TMEV model is uniquely positioned to identify and extend the preclinical profile of novel investigational therapies for the treatment and prevention of epilepsy, including SOT. This is primarily because the TMEV model is one of the few acquired epilepsy models available that relies on a naturally occurring rodent pathogen to evoke infection-induced acute seizures and epileptogenesis. It is unlike many other acquired epilepsy models in that it does not rely on a chemical or electrical insult to induce epileptogenesis (Barker-Haliski et al., 2017; Wilcox and Vezzani, 2014). Discrete inflammatory mechanisms associated with the cytokine storm and encephalitis itself likely contribute to the later onset of epilepsy and behavioral comorbidities, therefore the TMEV model is uniquely positioned to identify agents that both exert acute anticonvulsant, as well as anti-inflammatory or disease-modifying effects. Notably, agents that primarily exert anticonvulsant effects demonstrate mixed efficacy in the TMEV model, with some agents like carbamazepine exacerbating acute seizure incidence and chronic behavioral comorbidities long-term (Barker-Haliski et al., 2015). Because of its exquisite sensitivity and ability to identify agents that are either beneficial (e.g. minocycline (Barker-Haliski et al., 2016a; Libbey et al., 2016a)) or detrimental to disease progression (e.g. carbamazepine (Barker-Haliski et al., 2015), the TMEV model is useful to assess novel therapeutic mechanisms for epilepsy, including anti-inflammatory or disease-modifying agents (Barker-Haliski et al., 2016a; Libbey et al., 2016a). For example, pharmacological depletion of microglia with PLX5622 is both beneficial (Waltl et al., 2018) and detrimental in the TMEV model (Sanchez et al., 2019), highlighting conflicting effects of immune system modulation on acute disease severity and survival rate in the TMEV model. Cannabidiol (Patel et al., 2019) and TNF-alpha inhibition (Patel et al., 2017) have also demonstrated mixed effects against the acute behavioral seizures in this model. While the present study was not designed to assess acute or chronic changes in neuropathological features of acquired epilepsy resulting from CNS infection and SOT administration, (such as neuroinflammation or neurodegeneration), the effects of SOT administration on neuropathology should be established in future studies using this and other acquired epilepsy models. SOT administration herein demonstrated a clear anticonvulsant profile that led to improvements in chronic behavioral comorbidities in a mouse model of infection-induced epilepsy. As a result, the therapeutic novelty of SOT and breadth of anticonvulsant efficacy is confirmed and extended from prior investigations (Hawkins et al., 2021; Nishi et al., 2020, 2022). Future studies with the TMEV model of epilepsy could, as a result, be a strategy to further our understanding of the role of cholesterol 24-hydroxylase as a therapeutic target and identify other agents that meaningfully shift long-term disease outcomes post-infection to reduce the global burden of epilepsy.

CRediT authorship contribution statement

Melissa Barker-Haliski: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Toshiya Nishi:** Conceptualization, Methodology, Resources, Project administration, Writing – original draft, Writing – review & editing, Visualization. **H. Steve White:** Conceptualization, Methodology, Visualization, Supervision, Formal analysis, Investigation, Writing – review & editing.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by Takeda Pharmaceuticals.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2022.109310.

References

Alexandrov, P., Cui, J.G., Zhao, Y., Lukiw, W.J., 2005. 24S-hydroxycholesterol induces inflammatory gene expression in primary human neural cells. Neuroreport 16, 909–913.

Annegers, J.F., Hauser, W.A., Lee, J.R., Rocca, W.A., 1995. Incidence of acute symptomatic seizures in Rochester, Minnesota, 1935-1984. Epilepsia 36, 327–333.

Antunes, M., Biala, G., 2012. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cognit. Process. 13, 93–110.

Ba-Diop, A., Marin, B., Druet-Cabanac, M., Ngoungou, E.B., Newton, C.R., Preux, P.M., 2014. Epidemiology, causes, and treatment of epilepsy in sub-Saharan Africa. Lancet Neurol. 13, 1029–1044.

Barker-Haliski, M., Knox, K., Zierath, D.K., Koneval, Z., Metcalf, C.S., Wilcox, K.S., White, H.S., 2021. Development of an Antiepileptogenesis Drug Screening Platform: Effects of Everolimus and Phenobarbital. Epilepsia In press.

Barker-Haliski, M.L., Dahle, E.J., Heck, T.D., Pruess, T.H., Vanegas, F., Wilcox, K.S., White, H.S., 2015. Evaluating an etiologically relevant platform for therapy development for temporal lobe epilepsy: effects of carbamazepine and valproic acid on acute seizures and chronic behavioral comorbidities in the Theiler's murine encephalomyelitis virus mouse model. J. Pharmacol. Exp. Therapeut. 353, 318–329.

Barker-Haliski, M.L., Heck, T.D., Dahle, E.J., Vanegas, F., Pruess, T.H., Wilcox, K.S., White, H.S., 2016a. Acute treatment with minocycline, but not valproic acid, improves long-term behavioral outcomes in the Theiler's virus model of temporal lobe epilepsy. Epilepsia 57, 1958–1967.

Barker-Haliski, M.L., Loscher, W., White, H.S., Galanopoulou, A.S., 2017. Neuroinflammation in epileptogenesis: insights and translational perspectives from new models of epilepsy. Epilepsia 58 (Suppl. 3), 39–47.

Barker-Haliski, M.L., Vanegas, F., Mau, M.J., Underwood, T.K., White, H.S., 2016b. Acute cognitive impact of antiseizure drugs in naive rodents and corneal-kindled mice. Epilepsia 57, 1386–1397.
Barton, M.E., Peters, S.C., Shannon, H.E., 2003. Comparison of the effect of glutamate receptor modulators in the 6 Hz and maximal electroshock seizure models. Epilepsy Res. 56, 17–26.
Broer, S., Kaufer, C., Haist, V., Li, L., Gerhauser, I., Anjum, M., Bankstahl, M., Baumgartner, W., Loscher, W., 2016. Brain inflammation, neurodegeneration and seizure development following picornavirus infection markedly differ among virus and mouse strains and substrains. Exp. Neurol. 279, 57–74.
Carpio, A., Hauser, W., Aguirre, R., Roman, M., 2001. Etiology of epilepsy in Ecuador. Epilepsia 42.
Cohen, S.J., Stackman Jr., R.W., 2015. Assessing rodent hippocampal involvement in the novel object recognition task. A review. Behav. Brain Res. 285, 105–117.
Del Brutto, O.H., 2016. Diagnostic criteria for neurocysticercosis. Ann. Neurol. 80, 953–954.
Galanopoulou, A.S., Ferastraoaru, V., Correa, D.J., Cherian, K., Duberstein, S., Gursky, J., Hanumanthu, R., Hung, C., Molinero, I., Khodakivska, O., Legatt, A.D., Patel, P., Rosengard, J., Rubens, E., Sugrue, W., Yozawitz, E., Mehler, M.F., Ballaban-Gil, K., Haut, S.R., Moshe, S.L., Boro, A., 2020. EEG findings in acutely ill patients investigated for SARS-CoV-2/COVID-19: a small case series preliminary report. Epilepsia Open 5, 314–324.
Grayson, B., Leger, M., Piercy, C., Adamson, L., Harte, M., Neill, J.C., 2015. Assessment of disease-related cognitive impairments using the novel object recognition (NOR) task in rodents. Behav. Brain Res. 285, 176–193.
Hahn, C.D., Jiang, Y., Villanueva, V., Zolnowska, M., Arkilo, D., Hsiao, S., Asgharnejad, M., Dlugos, D., 2022. A phase 2, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of soticlestat as adjunctive therapy in pediatric patients with Dravet syndrome or Lennox-Gastaut syndrome (ELEKTRA). Epilepsia 63 (10), 2671–2683.
Halford, J.J., Sperling, M.R., Arkilo, D., Asgharnejad, M., Zinger, C., Xu, R., During, M., French, J.A., 2021. A phase 1b/2a study of soticlestat as adjunctive therapy in participants with developmental and/or epileptic encephalopathies. Epilepsy Res. 174, 106646.
Hammond, R.S., Tull, L.E., Stackman, R.W., 2004. On the delay-dependent involvement of the hippocampus in object recognition memory. Neurobiol. Learn. Mem. 82, 26–34.
Hawkins, N.A., Jurado, M., Thaxton, T.T., Duarte, S.E., Barse, L., Tatsukawa, T., Yamakawa, K., Nishi, T., Kondo, S., Miyamoto, M., Abrahams, B.S., During, M.J., Kearney, J.A., 2021. Soticlestat, a novel cholesterol 24-hydroxylase inhibitor, reduces seizures and premature death in Dravet syndrome mice. Epilepsia 62, 2845–2857.
Kilkenny, C., Browne, W., Cuthill, I.C., Emerson, M., Altman, D.G., 2010. Animal research: reporting in vivo experiments: the ARRIVE guidelines. Group, N. C. R. R. G. W. Br. J. Pharmacol. 160, 1577–1579.
Kirkman, N.J., Libbey, J.E., Wilcox, K.S., White, H.S., Fujinami, R.S., 2010. Innate but not adaptive immune responses contribute to behavioral seizures following viral infection. Epilepsia 51, 454–464.
Koike, T., Yoshikawa, M., Ando, H.K., Farnaby, W., Nishi, T., Watanabe, E., Yano, J., Miyamoto, M., Kondo, S., Ishii, T., Kuroita, T., 2021. Discovery of soticlestat, a potent and selective inhibitor for cholesterol 24-hydroxylase (CH24H). J. Med. Chem. 64, 12228–12244.
Libbey, J.E., Doty, D.J., Sim, J.T., Cusick, M.F., Round, J.L., Fujinami, R.S., 2016a. The effects of diet on the severity of central nervous system disease: one part of lab-to-lab variability. Nutrition 32, 877–883.
Libbey, J.E., Hanak, T.J., Doty, D.J., Wilcox, K.S., Fujinami, R.S., 2016b. NBQX, a highly selective competitive antagonist of AMPA and KA ionotropic glutamate receptors, increases seizures and mortality following picornavirus infection. Exp. Neurol. 280, 89–96.
Libbey, J.E., Kirkman, N.J., Smith, M.C., Tanaka, T., Wilcox, K.S., White, H.S., Fujinami, R.S., 2008. Seizures following picornavirus infection. Epilepsia 49, 1066–1074.
Loewen, J.L., Albertini, G., Dahle, E.J., Sato, H., Smolders, I.J., Massie, A., Wilcox, K.S., 2019. Genetic and pharmacological manipulation of glial glutamate transporters does not alter infection-induced seizure activity. Exp. Neurol. 318, 50–60.
Metcalf, C.S., Vanegas, F., Underwood, T., Johnson, K., West, P.J., Smith, M.D., Wilcox, K.S., 2022. Screening of prototype antiseizure and anti-inflammatory compounds in the Theiler's murine encephalomyelitis virus model of epilepsy. Epilepsia Open 7, 46–58.
Misra, U.K., Tan, C.T., Kalita, J., 2008. Viral encephalitis and epilepsy. Epilepsia 49 (6), 13–18.
Nishi, T., Kondo, S., Miyamoto, M., Watanabe, S., Hasegawa, S., Kondo, S., Yano, J., Watanabe, E., Ishi, T., Yoshikawa, M., Ando, H.K., Farnaby, W., Fujimoto, S., Sunahara, E., Ohori, M., During, M.J., Kuroita, T., Koike, T., 2020. Soticlestat, a novel cholesterol 24-hydroxylase inhibitor shows a therapeutic potential for neural hyperexcitation in mice. Sci. Rep. 10, 17081.
Nishi, T., Metcalf, C.S., Fujimoto, S., Hasegawa, S., Miyamoto, M., Sunahara, E., Watanabe, S., Kondo, S., White, H.S., 2022. Anticonvulsive properties of soticlestat, a novel cholesterol 24-hydroxylase inhibitor. Epilepsia 63, 1580–1590.
Patel, D.C., Wallis, G., Dahle, E.J., McElroy, P.B., Thomson, K.E., Tesi, R.J., Szymkowski, D.E., West, P.J., Smeal, R.M., Patel, M., Fujinami, R.S., White, H.S., Wilcox, K.S., 2017. Hippocampal TNFalpha signaling contributes to seizure generation in an infection-induced mouse model of limbic epilepsy. eNeuro 4.
Patel, D.C., Wallis, G., Fujinami, R.S., Wilcox, K.S., Smith, M.D., 2019. Cannabidiol reduces seizures following CNS infection with Theiler's murine encephalomyelitis virus. Epilepsia Open 4, 431–442.
Paul, S.M., Doherty, J.J., Robichaud, A.J., Belfort, G.M., Chow, B.Y., Hammond, R.S., Crawford, D.C., Linsenbardt, A.J., Shu, H.J., Izumi, Y., Mennerick, S.J., Zorumski, C. F., 2013. The major brain cholesterol metabolite 24(S)-hydroxycholesterol is a potent allosteric modulator of N-methyl-D-aspartate receptors. J. Neurosci. 33, 17290–17300.
Praag, H., Dreyfus, C.F., Black, I.B., 1994. Dissociation of motor hyperactivity and spatial memory deficits by selective hippocampal lesions in the neonatal rat. J. Cognit. Neurosci. 6, 321–331.
Sanchez, J.M.S., DePaula-Silva, A.B., Doty, D.J., Truong, A., Libbey, J.E., Fujinami, R.S., 2019. Microglial cell depletion is fatal with low level picornavirus infection of the central nervous system. J. Neurovirol. 25, 415–421.
Singh, G., Prabhakar, S., Modi, M., 2008. Central nervous system infections and epilepsy. Epilepsia 49 (6), 1.
Singh, G., Sander, J.W., 2020. The global burden of epilepsy report: implications for low- and middle-income countries. Epilepsy Behav. 105, 106949.
Stewart, K.A., Wilcox, K.S., Fujinami, R.S., White, H.S., 2010a. Development of postinfection epilepsy after Theiler's virus infection of C57BL/6 mice. J. Neuropathol. Exp. Neurol. 69, 1210–1219.
Stewart, K.A., Wilcox, K.S., Fujinami, R.S., White, H.S., 2010b. Theiler's virus infection chronically alters seizure susceptibility. Epilepsia 51, 1418–1428.
Umpierre, A.D., Remigio, G.J., Dahle, E.J., Bradford, K., Alex, A.B., Smith, M.D., West, P. J., White, H.S., Wilcox, K.S., 2014. Impaired cognitive ability and anxiety-like behavior following acute seizures in the Theiler's virus model of temporal lobe epilepsy. Neurobiol. Dis. 64, 98–106.
Walker, J.M., Fowler, S.W., Miller, D.K., Sun, A.Y., Weisman, G.A., Wood, W.G., Sun, G. Y., Simonyi, A., Schachtman, T.R., 2011. Spatial learning and memory impairment and increased locomotion in a transgenic amyloid precursor protein mouse model of Alzheimer's disease. Behav. Brain Res. 222, 169–175.
Waltl, I., Kaufer, C., Gerhauser, I., Chhatbar, C., Ghita, L., Kalinke, U., Loscher, W., 2018. Microglia have a protective role in viral encephalitis-induced seizure development and hippocampal damage. Brain Behav. Immun. 74, 186–204.
WHO, 2022. In: Organization, W.H. (Ed.), Epilepsy Fact Sheet.
Wilcox, K.S., Vezzani, A., 2014. Does brain inflammation mediate pathological outcomes in epilepsy? Adv. Exp. Med. Biol. 813, 169–183.