Sodium channel inhibition with GS967 improves survival and suppresses spontaneous seizures in Dravet syndrome mice

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ABSTRACT

Dravet syndrome is a catastrophic pediatric epileptic encephalopathy in which patients suffer from severe spontaneous seizures and develop comorbidities including cognitive impairment, gait disturbances and psychomotor dysfunction. Dravet syndrome responds poorly to currently available antiepileptic drugs and exhibits an unfavorable long-term survival. More than 70% of Dravet syndrome patients have de novo heterozygous missense or truncation mutations in SCN1A suggesting haplosufficiency of SCN1A as the genetic mechanism. Loss-of-function Dravet syndrome has been modeled by Scn1a knockout (Scn1a−/−) mice, which mimic the severe epilepsy phenotype. Studies using Scn1a−/− mice have demonstrated impaired excitability in GABAergic interneurons and have led to the prevailing hypothesis that impaired GABA-mediated inhibition is responsible for epileptogenesis in Dravet syndrome. Clinical observations suggest that conventional sodium channel blocking anticonvulsants are not generally effective in patients with Dravet syndrome and may cause a worsening of the disease. We hypothesized that GS967, an unconventional sodium channel blocker that exhibits anticonvulsant activity in transgenic mice expressing a gain-of-function sodium channel mutation, would likely exacerbate the phenotype of Scn1a−/− mice. GS967 is known to exert preferential inhibition of persistent sodium current in cells transfected with mutant brain sodium channels Na1,1 and Na1,2, as well as neurons expressing a gain-of-function Na1,2 mutation. GS967 treatment had no effect on temperature threshold of hyperthermia-induced seizures in Scn1a−/− mice assessed at P14–P18. However, unexpectedly, GS967 treated Scn1a−/− mice had no seizures over a 48 hour period compared to a total of 52 seizures in untreated mice (n=10–12, p = 0.01). Additionally, GS967 treatment significantly improved survival of Scn1a−/− mice compared to untreated animals (n=16–18, p = 0.001). Whole-cell voltage clamp experiments on acutely dissociated pyramidal neurons revealed that GS967 exerted profound effects on sodium channel inactivation by shifting the voltage dependence of inactivation, slowing recovery from fast inactivation, enhancing entry into slow inactivation, and exhibiting strong use-dependent block. Our findings suggest that the unconventional sodium channel blocker GS967 exerts favorable effects on survival and spontaneous seizure frequency in a mouse model of Dravet syndrome.

METHODS

Mice - Scn1a−/− mice were maintained on a congenic 129S6/SvEvTac background (129S6-Scn1a−/−). A cross between wildtype C57BL/6J (B6) and 129S6-Scn1a−/− mice yields heterozygotes on the F1 background (F1-Scn1a−/−).

Anticonvulsant Testing - We used male and female F1-Scn1a−/− mice in these experiments. GS967 was administered orally through supplementation in chow (9 mg GS967/kg chow; dosage estimated as 1.5 mg/kg/day based on the consumption of 3.5–4 g chow per 24 h) for 24 h. Drug treatment began at postnatal day 18 to 24 by quantifying the total number of behavioral seizures over 48 hours in untreated and treated Scn1a−/− mice. Statistical comparisons were made using Fisher’s exact test.

Hyperthermia-induced Seizure Testing - Experiments were done in F1-Scn1a−/− mice postnatal day 14 to 18. Prior to the induction of hyperthermic seizures, GS967 was administered orally through supplementation in chow beginning at postnatal day 10. Previous experiments demonstrated that GS967 is efficiently transmitted from lactating dams to nursing pups. A heat lamp was used to elevate the mouse core body temperature 0.5° every 2 minutes. Body temperature was monitored using a rectal probe and mice were continuously monitored for the onset of the first convulsion with loss of posture or until 40°C was reached. Statistical comparisons were made using log-rank test.

Electrophysiology - Hippocampal pyramidal neurons were isolated from F1-Scn1a−/− mice postnatal day 21–24. Hippocampi were dissected from 400 µM thick coronal brain slices and placed in recording solution containing 1 mg/ml bovine serum albumin, then placed in recording solution for electrophysiological experiments. Whole cell voltage clamp recordings were performed as previously described (Thompson et al., Epilepsia, 2011). Electrophysiological recordings were performed in the absence or presence of 2 µM GS967 which is the concentration in the brains of treated mice. Statistical comparisons were made using Student’s t-test.

Western blot analysis - Western blot analysis was performed on hippocampal membrane preparations that were isolated by differential centrifugation from postnatal day 23 untreated and GS967 treated Scn1a−/− mice. Membrane proteins were subjected to 7% SDS-PAGE and transferred to a PVDF membrane. Proteins were detected with primary antibodies directed against Na1.6 (rabbit, anti-Na1.6 polyclonal, 1:1500, Abcam Ltd.) or the loading control β-tubulin (mouse, anti-β-tubulin clone TUB1.1, 1:5000, Sigma-Aldrich). Immunoreactive bands were detected on an Odyssey imager using fluorescent secondary antibodies directed at the primary antibodies (goat, anti-rabbit 600 or anti-mouse 680, LI-COR, Inc.). Densitometrically quantified bands were normalized to that of β-tubulin. Statistical comparisons were made using Student’s t-test.

CONCLUSION

GS967, an unconventional sodium channel inhibitor, exhibits antiepileptic properties in Dravet syndrome mice. These positive outcomes may result from GS967 acutely stabilizing channel inactivation and chronically downregulating expression of Na1.6, thereby preventing neuronal hyperexcitability associated with seizures. Based upon divergent treatment responses, we also conclude that spontaneous seizures and hyperthermia-induced seizures may have different mechanisms in Dravet syndrome.

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