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BRIEF COMMUNICATION

Donepezil increases resistance to induced seizures in a mouse model of Dravet syndrome

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Abstract

De novo loss-of-function mutations in *SCN1A* are the main cause of Dravet syndrome, a catastrophic encephalopathy characterized by recurrent early-life febrile seizures, a number of other afebrile seizure types that are often refractory to treatment, and behavioral abnormalities including social deficits, motor dysfunction, and cognitive impairment. We previously demonstrated that the reversible acetylcholinesterase inhibitor, Huperzine A, increases seizure resistance in *Scn1a* mutants. In the present study, we evaluated the therapeutic potential of donepezil, a reversible acetylcholinesterase inhibitor approved by the Food and Drug Administration, in a mouse model of Dravet syndrome ($Scn1a^{+/-}$). We found that donepezil conferred robust protection against induced seizures in $Scn1a^{+/-}$ mutants.

Introduction

Reversible acetylcholinesterase (AChE) inhibitors (e.g., donepezil, galantamine, and rivastigmine) are most widely used in the treatment of dementia and Alzheimer's disease (AD).[1] Donepezil, a synthetic reversible AChE inhibitor, also shows efficacy in rodent models of AD,[2,3] neuropathic pain,[4] and autism.[5] In rodent studies, donepezil is more effective at penetrating the brain and has slower brain clearance compared to galantamine,[6] resulting in longer lasting effects. In addition, donepezil primarily inhibits AChE, whereas galantamine lacks specificity and can modulate other neurotransmitters (e.g., glutamate, dopamine, serotonin).[7]

There is evidence to suggest that reversible AChE inhibitors might also be beneficial in the treatment of epilepsy. Donepezil, galantamine, and Huperzine A (Hup A), a naturally occurring reversible AChE inhibitor, have been shown to protect against soman-induced seizures.[8–13] Hup A also provides protection against N-methyl-D-aspartate (NMDA)-induced status epilepticus (SE)[14] and pentylenetetrazole (PTZ)-induced seizures in rats[15] and zebrafish.[16] In addition, we recently demonstrated that Hup A confers robust protection against induced seizures in $Scn1a^{+/-}$ and $Scn1a^{RH/+}$ mouse models of Dravet syndrome (DS) and genetic epilepsy with febrile seizures plus (GEFS+), respectively.[17] Since Hup A effectively increased seizure resistance, we hypothesize that other reversible AChE inhibitors, such as donepezil, may also be efficacious in the treatment of epilepsy.

Although the effect of donepezil on spontaneous seizures was not examined, it was recently demonstrated that administration of donepezil following pilocarpine-induced SE protected against hippocampal neuron loss.[18] Furthermore, donepezil also protects against glutamate neurotoxicity.[19] Although the primary goal in the clinical management of epilepsy is often the prevention of seizures, patients with epilepsy often have cognitive and behavioral abnormalities that can significantly impact quality of life. Donepezil has been shown to improve

social preference and increase social interaction in a mouse model of autism,[5] suggesting that it could also potentially ameliorate behavioral comorbidities in epilepsy. The few studies that have evaluated the effect of donepezil on memory in patients with epilepsy have been inconsistent. For example, in patients with partial seizures, donepezil (10 mg/day for 2 months) was shown to improve word recall[20]; however, Hamberger et al. found no effect of donepezil (10 mg/day for 3 months) on memory in epileptic patients[21]. Moreover, both studies reported mild gastrointestinal issues and insomnia, but importantly, no significant increase in seizure frequency.[20,21]

De novo loss-of-function mutations in the voltage-gated sodium channel (VGSC) *SCN1A* (encoding $Na_v1.1$) are the main cause of DS (OMIM 607208), an early-life encephalopathy characterized by recurrent early-life febrile seizures (FSs), and several other seizure types, including generalized tonic-clonic (GTCS), myoclonic, and partial seizures. These seizures are often refractory to treatment. Furthermore, patients with DS often have comorbid abnormalities, including motor dysfunction, social deficits, and cognitive impairment.[22–24] *SCN1A* mutations also lead to GEFS+, which is an inherited disorder characterized by FSs that persist beyond early childhood and the development of adult epilepsy.[25,26] We and others have shown that mice with *Scn1a* mutations recapitulate many clinical features, including the development of spontaneous seizures.[27,28] In the current study, we evaluated the therapeutic potential of donepezil in a mouse model of DS.

Materials and Methods

Animals

Male CF1 mice (Strain: 023, Charles River, 2–3 months old) were used to generate a dose-response curve based on seizures induced by the 6 Hz seizure induction paradigm. CF1 mice were also used to test the contribution of muscarinic and $GABA_A$ receptors to the observed donepezil-mediated seizure protection using the 6 Hz paradigm. Heterozygous *Scn1a* knockout mice ($Scn1a^{+/-}$) were generated as previously described[27] and maintained by backcrossing to FVB/NJ (Strain: 001800, Jackson Laboratories). Experimental $Scn1a^{+/-}$ mutants and wild-type (WT) littermates were generated by crossing once to the C57BL/6J background. Male and female $Scn1a^{+/-}$ mutants and WT littermates (p21–p23) were used for hyperthermia seizure induction. Male $Scn1a^{+/-}$ mutants and WT littermates (4–6 weeks old) were used for 6 Hz and PTZ seizure induction. Male and female $Scn1a^{+/-}$ mutants and WT littermates (4–6 weeks old) were used for maximal electroshock (MES) seizure induction. The AChE assay was performed on male WT littermates (2 months old). All mice were housed on a 12-h light/dark cycle with food and water ad libitum. All experiments were performed in accordance with the guidelines of the Institutional of Animal Care and Use Committee of Emory University. The experimenter was blinded to genotype; however, since donepezil administration results in transient visible mild side effects, it was not possible for the experimenter to be blinded to treatment.

Donepezil administration

Donepezil hydrochloride (Sigma-Aldrich) was dissolved in sterile saline (0.9%). For acute administration, donepezil (10 mL/kg) or vehicle (0.9 % sterile saline) was administered via intraperitoneal (i.p.) injection 1 h prior to seizure induction. See supplemental for additional methods.

Results

Donepezil protects against induced seizures in CF1 mice

We first evaluated the effect of donepezil (1–10 mg/kg) on 6 Hz-induced seizures (44 mA) in male CF1 WT mice ($N = 10$/group). All vehicle-treated mice ($N = 10$) exhibited 6 Hz seizures consisting of forelimb clonus and paw waving (RS2 seizures). However, administration of 5.6 and 10 mg/kg donepezil significantly reduced seizure occurrence, whereby 4/10 and 5/10 treated mice were completely protected against 6 Hz-induced seizures, respectively (Fig. 1A). However, significant adverse effects (lethargy, tremor, and approximately 30% mortality) were observed at 10 mg/kg donepezil; therefore, the 5.6 mg/kg dose was used for all subsequent experiments. The ability of donepezil (5.6 mg/kg) to protect against MES-induced seizures was also examined ($N = 10$/group, Fig. 1B). Maximal seizures characterized by hindlimb extension were observed in all vehicle-treated mice following MES induction; however, 80% (8/10) donepezil-treated mice were protected against MES-induced hindlimb extension.

Central muscarinic and $GABA_A$ receptors contribute to the observed donepezil-mediated seizure protection

To gain insight into the potential mechanism(s) by which donepezil mediates seizure protection, we coadministered donepezil and either a muscarinic or $GABA_A$ receptor antagonist prior to 6 Hz seizure induction in CF1 mice (Fig. 1C and D). These two antagonists were selected as we previously showed that Hup A-mediated seizure

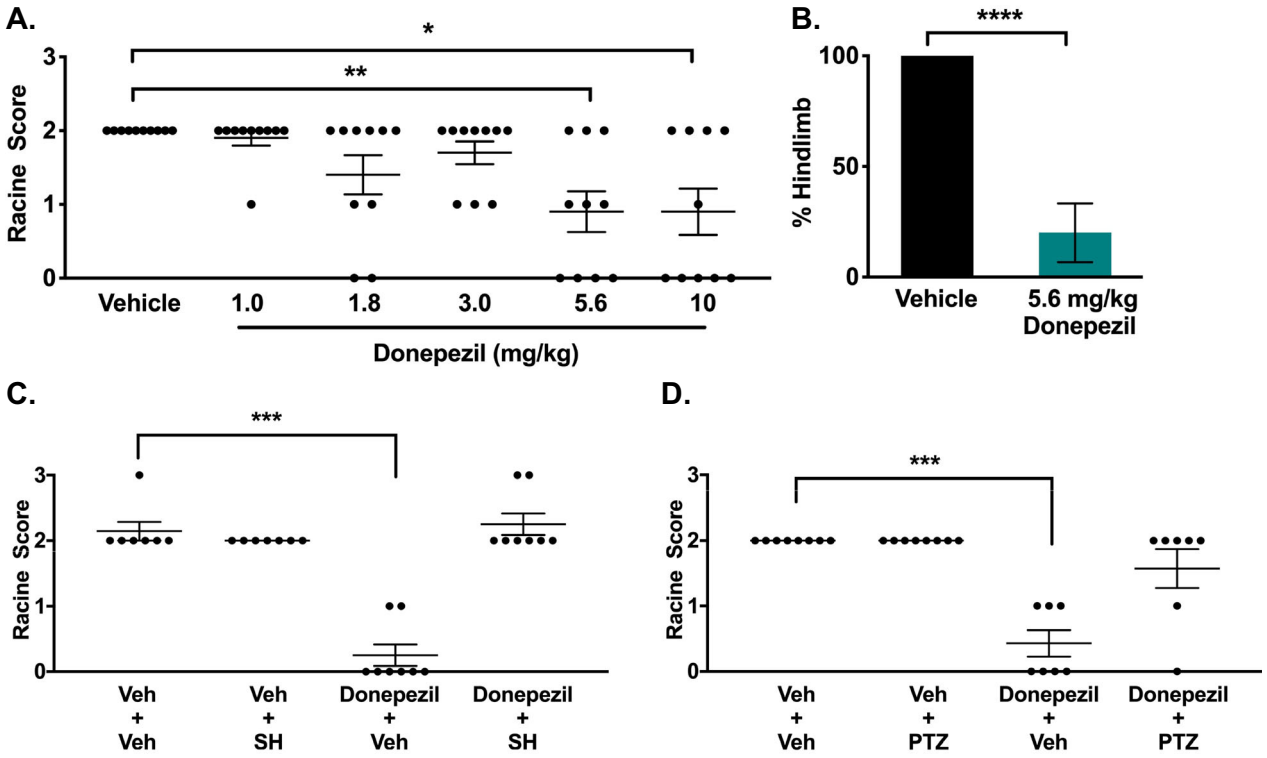

Figure 1. Donepezil confers robust protection against 6 Hz- and MES-induced seizures in CF1 mice. (A) A ¼ log dose-response curve was generated to determine the relationship between donepezil administration and resistance to 6 Hz-induced seizures in CF1 WT mice (N = 10/dose). Donepezil or vehicle was administered 1 h prior to seizure induction (44 mA). The greatest protection against 6 Hz-induced seizures was observed at 5.6 and 10 mg/kg donepezil. One-way ANOVA followed by Dunn's multiple comparisons post hoc analysis. (B) Donepezil (5.6 mg/kg) significantly increased resistance to MES-induced seizures in CF1 mice (N = 10/group). Unpaired student's t-test. (C) The muscarinic receptor antagonist, scopolamine hydrobromide (SH), blocks the ability of donepezil to protect against 6 Hz-induced seizures. N = 7–8/group. (D) Block of the $GABA_A$ receptor by administration of pentylenetetrazole (PTZ) also abolishes the donepezil-mediated protection. One-way ANOVA followed by Dunn's multiple comparisons post hoc analysis. N = 7–8/group. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

protection works, in part, through these two receptors[17]. Control mice were similarly handled and administered two injections of vehicle. Block of central muscarinic receptors was achieved by administration of scopolamine hydrobromide (SH, Fig. 1C). Behavioral seizure responses were comparable between control mice that received vehicle and mice that received SH plus vehicle, demonstrating that SH alone does not alter the behavioral seizure response (Fig. 1C). Seventy-five percent (6/8) of mice administered donepezil plus vehicle were completely protected against 6 Hz-induced seizures (6 RS0, 2 RS1, Fig. 1C). In contrast, all mice administered donepezil plus SH exhibited seizures (6 RS2, 2 RS3, Fig. 1C), demonstrating that centrally located muscarinic receptors contribute to donepezil-mediated seizure protection. Control mice administered PTZ (25 mg/kg) plus vehicle exhibited comparable seizure responses to mice that received only vehicle, demonstrating that this dose of PTZ does not alter the seizure response (Fig. 1D). Only 1/8 mice administered donepezil plus PTZ was protected against 6 Hz-induced seizures (1 RS0, 1 RS1, 6 RS2), demonstrating that donepezil-mediated seizure protection also works, in part, by activation of $GABA_A$ receptors.

Donepezil increases resistance to induced seizures in *Scn1a*$^{+/-}$ mutants

Since we observed seizure protection in CF1 WT mice, we next tested donepezil in *Scn1a*$^{+/-}$ mutants and WT littermates. We first determined the effect of 5.6 and 10 mg/kg donepezil on brain AChE activity in the WT littermates 1 h after administration. When compared to vehicle-treated mice, we observed 21% and 46% reduction in AChE activity with 5.6 and 10 mg/kg donepezil, respectively (N = 3–4/group, Fig. 2A).

We next evaluated the effect of donepezil against induced seizures in *Scn1a*$^{+/-}$ mutants and WT littermates. Mice were administered donepezil (5.6 mg/kg) or vehicle

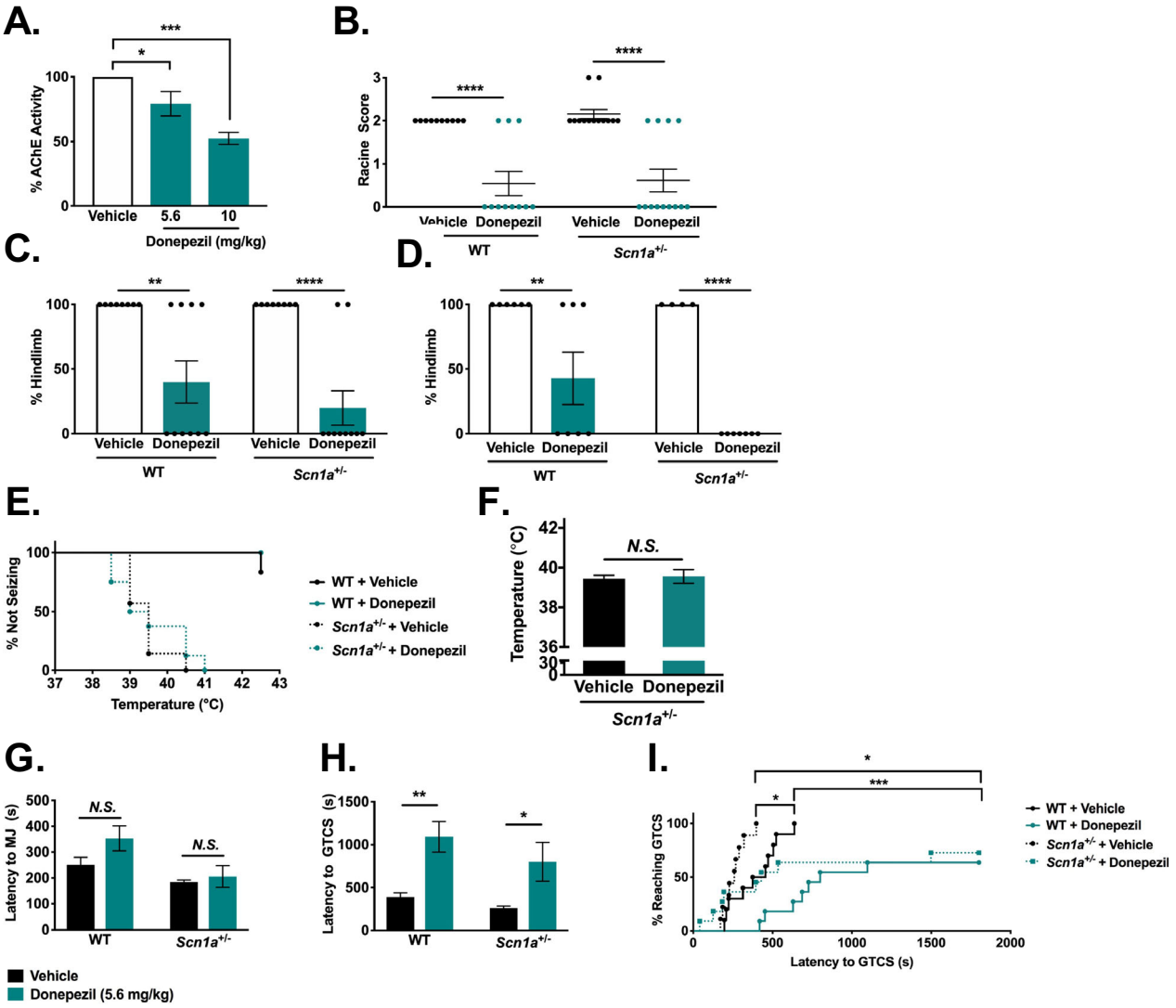

Figure 2. Donepezil increases resistance to induced seizures in *Scn1a*$^{+/-}$ mutant mice and WT littermates. (A) Donepezil reduces AChE activity by 21% and 46% at doses of 5.6 and 10 mg/kg, respectively (N = 3–4/group). One-way ANOVA followed by Dunnett's multiple comparisons post hoc analysis. (B) Donepezil increases resistance to 6 Hz-induced seizures (20 mA) in *Scn1a*$^{+/-}$ mutants and WT littermates (N = 10–13/genotype/treatment). One-way rANOVA followed by Dunn's multiple comparisons post hoc analysis. (C–D) Donepezil increases resistance to MES-induced seizures in male (C) and female (D) *Scn1a*$^{+/-}$ mutants and WT littermates (males, N = 4–7/genotype/treatment, females, N = 8–10/genotype/treatment). Two-way ANOVA followed by Holm-Šídák's multiple comparisons test. (E) Donepezil administration did not protect against hyperthermia-induced seizures in *Scn1a*$^{+/-}$ mutants and WT littermates (N = 5–8/genotype/treatment). (F) The average temperature at which *Scn1a*$^{+/-}$ mutants seized following hyperthermia induction was comparable between vehicle- and donepezil-treated mutants. (G) Donepezil- and vehicle-treated mice had comparable latencies to the first myoclonic jerk following PTZ induction. (H) Donepezil significantly increased the latency to the first generalized tonic-clonic seizure after PTZ induction (N = 10–11/genotype/treatment). Two-way ANOVA followed by Holms-Šídák's multiple comparisons test. (I) Approximately 50% of donepezil-treated mice, regardless of genotype, did not exhibit a PTZ-induced GTCS, whereas all vehicle-treated mice exhibited a GTCS. Two-way ANOVA followed by Holms-Šídák's multiple comparisons test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

1 h prior to seizure induction. With the 6 Hz seizure induction paradigm (Fig. 2B), seizures were observed in all vehicle-treated mice (WT, 10 RS2; *Scn1a*$^{+/-}$, 11 RS2, 2 RS3). Following the administration of donepezil, 8/11 WT littermates were completely protected against 6 Hz-induced seizures. Similarly, 9/13 donepezil-treated *Scn1a*$^{+/-}$ mutants were protected (9 RS0, 4 RS2). We next assessed the ability of donepezil to protect against MES-induced seizures in both sexes of *Scn1a*$^{+/-}$ mutants and WT littermates (Fig. 2C and D). As expected, all vehicle-treated

mice, regardless of sex and genotype, exhibited a maximal seizure characterized by hindlimb extension. Following the administration of donepezil, protection was observed in 4/10 and 7/7 male WT littermates and *Scn1a*$^{+/-}$ mutants, respectively (Fig. 2C). Similarly, protection against hindlimb extension was observed in 6/10 and 8/10 donepezil-treated female WT littermates and *Scn1a*$^{+/-}$ mutants, respectively (Fig. 2D).

Febrile seizures are commonly observed in patients with *SCN1A*-derived epilepsy. FS susceptibility in *Scn1a* mutant mice was examined by elevating the core body temperature of each mouse until the first GTCS was observed. We observed no difference in the temperature at which the *Scn1a*$^{+/-}$ mutant mice exhibited seizures following donepezil or vehicle administration (Fig. 2E and F).

Finally, we evaluated the ability of donepezil to protect against PTZ-induced seizures. We observed no significant effect of donepezil on the latency to the first myoclonic jerk in *Scn1a*$^{+/-}$ mutants and WT littermates following PTZ administration (100mg/kg, Fig. 2G). However, donepezil-treated WT littermates and *Scn1a*$^{+/-}$ mutants exhibited significantly increased latencies to the first GTCS (Fig. 2H and I). Furthermore, 4/11 and 3/11 donepezil-treated WT littermates and *Scn1a*$^{+/-}$ mutants did not exhibit a GTCS, demonstrating that donepezil provides robust protection against PTZ-induced seizures.

Discussion

Recent evidence suggests that reversible AChE inhibitors, such as Huperzine A and donepezil, may be therapeutic in epilepsy as evidenced by their ability to increase resistance to induced seizures[8–11,15,17]. When compared to Hup A, donepezil similarly conferred robust protection against 6 Hz-, MES-, and PTZ-induced seizures. However, unlike Hup A, donepezil was not able to increase resistance to hyperthermia-induced seizures in *Scn1a*$^{+/-}$ mutants.

Several observations suggest that the relative protective effects of donepezil and Hup A are not solely due to the reduction in AChE activity. First, we found that the highest levels of seizure protection were achieved at doses of 5.6 mg/kg donepezil and 1 mg/kg Hup A, which decreased AChE activity by 21% and 70%[17], respectively. Second, we observed 30% mortality with 10 mg/kg donepezil, which reduced AChE activity by 46%. However, no mortality was observed when Hup A was administered at 1 mg/kg. Third, lower doses of Hup A (0.1 and 0.18 mg/kg) reduced AChE activity by approximately 30%; however, these doses of Hup A did not provide protection against induced seizures[17]. Finally, we previously demonstrated that Hup A-mediated seizure protection is also regulated by activation of muscarinic cholinergic receptors, and in part, by $GABA_A$ receptors[17]. Consistent with this, we also observed that the protection following donepezil administration relies on contribution of both the muscarinic cholinergic and $GABA_A$ receptors.

In summary, we have demonstrated the ability of donepezil to increase seizure resistance in *SCN1A*-derived epilepsy. Together with our previous findings with Hup A, these results provide additional support that reversible AChE inhibitors might be efficacious in *SCN1A*-derived epilepsy and possibly other forms of refractory epilepsy.

Acknowledgments

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Conflict of Interest

None of the authors have any 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.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Description of each seizure induction paradigm and acetylcholinesterase assay.

