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BRIEF COMMUNICATION

Epilepsia®

Perampanel and decanoic acid show synergistic action against AMPA receptors and seizures

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Summary

Perampanel is an adjunctive treatment for epilepsy that works through the direct inhibition of AMPA receptors. The same molecular mechanism has recently been shown for a fatty acid, decanoic acid, prescribed in the medium chain triglyceride ketogenic diet for the treatment of patients with drug-resistant epilepsy. Because each compound has been proposed to act through a distinct AMPA receptor binding site, we predicted that perampanel and decanoic acid would act synergistically against AMPA receptors and, consequently, seizures. Here, we show a synergistic interaction between perampanel and decanoic acid in direct AMPA receptor inhibition, in an ex vivo model of seizure activity, and against seizure-induced activity in human brain slices. These data support a potential role for combination treatment using perampanel and dietary decanoic acid to provide enhanced seizure control.

KEYWORDS

AMPA receptors, decanoic acid, drug-resistant epilepsy, perampanel, synergy

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1 | INTRODUCTION

Perampanel (Fycompa) is a noncompetitive AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor antagonist and is an adjunctive treatment for partial-onset and primary generalized tonic–clonic seizures.[1] However, it has dose-dependent behavioral side effects, thereby limiting its use in some patients.[2] Although the binding site for perampanel has been well characterized on AMPA receptors,[3] there is little information on its subtype specificity and potential synergistic action with other AMPA receptor antagonists.

The medium chain triglyceride (MCT) ketogenic diet provides a nonpharmacologic treatment for patients with drug-resistant epilepsy.[4] Ketone generation was considered necessary for seizure control[5]; however, recent studies have shown a direct antiseizure effect of medium chain fatty acids,[6–8] likely to be through noncompetitive inhibition of AMPA receptors by decanoic acid (DA).[6] Because in silico analysis suggests that DA binds to the AMPA receptor at a site distinct to that of perampanel,[8] we hypothesized that combined perampanel and DA dosing will result in synergistic inhibition of AMPA receptors and enhanced seizure control.

2 | METHODS

2.1 | AMPA receptor oocyte electrophysiology

Recordings of recombinant AMPA receptor inward currents from Xenopus oocytes have been described previously.[8] Perampanel was purchased from Apexmol Technology Co. Ltd. (Beijing, China).

2.2 | Rat Hippocampal pentylenetetrazole seizure-like activity analysis

Seizure-like activity was induced in rat entorhinal cortex-hippocampal slices as described previously[8]; perampanel (0, 100, or 500 nmol/L) and DA (increasing concentrations at 10 minute intervals) were then applied. The frequency of the discharges was measured at minute intervals, averaged every 5 minutes, and normalized to baseline. IC_{50} values (half maximal inhibitory concentration) were calculated using a Hill plot with statistical analysis by one-way analysis of variance (ANOVA) with Dunnett's post hoc test.

2.3 | In vitro human brain slice studies

Neocortical human brain slices were derived from patients undergoing elective neurosurgical resection of a neoplasm, where 7 of 8 had a history of epileptic seizures. Preoperative informed consent was obtained for the use of resected brain tissue. This study was approved by the Newcastle and North Tyneside 2 Research Ethics Committee (06/Q1003/51), with clinical governance approved by the Newcastle Upon Tyne Hospitals National Health Service (NHS) Trust (CM/PB/3707). A human neocortical slice (450 μm) preparation was used as has been described previously.[9] Statistical analysis was performed using a Mann-Whitney test.

3 | RESULTS

3.1 | Direct inhibition of AMPA receptors by perampanel and decanoic acid

Prior to the investigation of a synergistic effect between perampanel and decanoic acid, we first investigated the effect of perampanel on currents elicited by glutamate (100 μmol/L) application to *Xenopus laevis* oocytes expressing 2 AMPA receptor subunit combinations, GluA1/2 or 2/3. These 2 subunit combinations are the most commonly found combinations in the hippocampus.[9] Perampanel inhibited AMPA receptor currents with an IC_{50} of 8.11 μmol/L against GluA2/3 (SEM [standard error of the mean] 2.05) and 6.27 μmol/L against GluA1/2 (SEM 1.02) (Figure S1A,B) in a noncompetitive manner (Figure S1C). Perampanel reduced maximal responses to 75.2% (at 2.5 μmol/L) and 16.9% (at 5 μmol/L) showing, for the first

time, the direct inhibition of AMPA receptors by perampanel through noncompetitive inhibition without subunit specificity.

Recent studies propose distinct binding sites for perampanel and DA on AMPA receptors,[3,6] with perampanel binding at the S1-M1 and S2-M4 linker[3] and DA binding to the M3 region.[6] To confirm this, we expressed a GluA3 mutant resistant to the AMPA receptor antagonist GYKI,[3] and assessed it for sensitivity to perampanel and DA (Figure S1D). Perampanel (20 μmol/L) reduced the wild-type AMPA receptor (GluA3) glutamate-induced currents by 94.7%, but the mutant receptor currents by only 54.3% ($P < 0.0001$). In contrast, DA (1 mmol/L) reduced both currents by 74.5% (SEM 6.4) and 72.8% (SEM 0.3), respectively, strongly supporting the proposed distinct sites of action.

The existence of distinct AMPA receptor binding sites for DA and perampanel raises the possibility of synergism; therefore we tested AMPA receptor sensitivity to DA at 2 concentrations of perampanel (1 and 4 μmol/L) (Figure 1A, B,C). For GluA2/3, the IC_{50} for DA decreased from 0.52 mmol/L (no perampanel) to 0.15 mmol/L (1 μmol/L perampanel) or 0.12 mmol/L (4 μmol/L perampanel) ($P < 0.0001$). For GluA1/2 the IC_{50} for DA decreased from 1.16 mmol/L (no perampanel) to 0.21 mmol/L (1 μmol/L perampanel) or 0.10 mmol/L (4 μmol/L perampanel) ($P < 0.0001$). Repeating this approach using perampanel with 2 concentrations of DA (50 and 100 μmol/L) (Figure 1D,E,F) also showed a significant increase in the potency of perampanel, decreasing IC_{50} values from 8.1 μmol/L (no DA) to 1.7 and 1.6 μmol/L in the presence of DA at 50 and 100 μmol/L, respectively, in GluA2/3 and from 6.2 μmol/L in GluA1/2 to 2.1 and 2.2 μmol/L, respectively (both $P < 0.0001$). These results imply synergistic inhibition of AMPA receptors by DA and perampanel.

To validate a synergistic effect of both perampanel and DA on AMPA receptor currents, an isobolographic analysis was used. This approach can distinguish between additive and synergistic effects of 2 compounds with similar effects (Figure 1G,H). In this analysis, the isobolic line represents additive effects of the compounds against both GluA1/2 and GluA2/3 receptors. From results provided here, combinatory treatment with perampanel and DA provides points at fixed ratios of each compound that lie below the isobolic line, indicating a synergistic effect. This analysis, therefore, supports a direct synergistic effect of perampanel and DA against AMPA receptors.

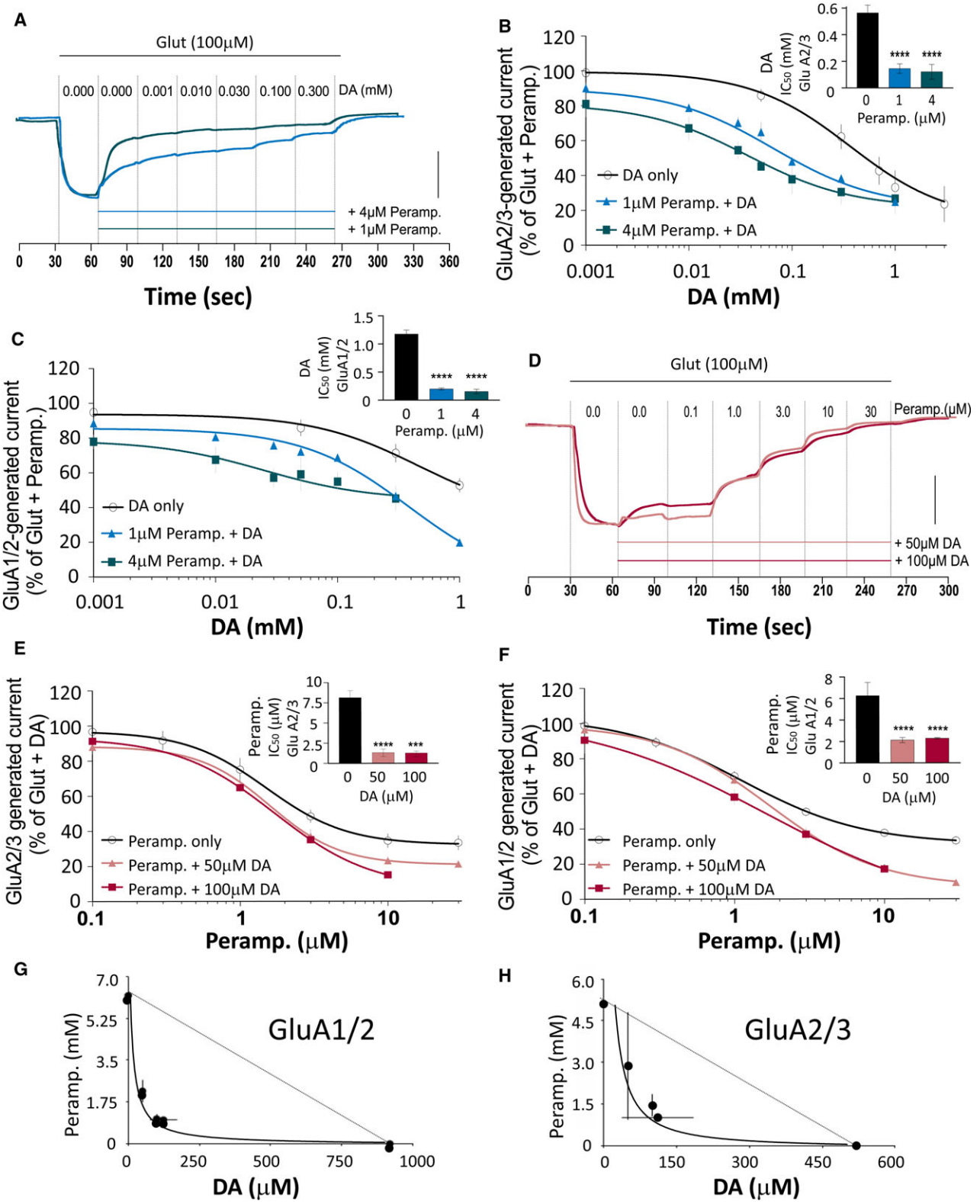
3.2 | Effects of perampanel and decanoic acid on an ex vivo (animal) seizure model

To investigate a synergistic effect in controlling seizure-like activity and to avoid the confounders of systemic metabolism or pharmacokinetic interactions, we then investigated the effects of combinatory perampanel and DA on PTZ-induced seizure-like activity in rat hippocampal slices. In these experiments, increasing DA concentrations reduced epileptiform activity at 300 μmol/L with a block at 1000 μmol/L (Figure 2A,B), consistent with earlier data.[6] Repeating of the assay with added perampanel (100 and 500 nmol/L) with data normalized to perampanel alone, showed that DA (10 μmol/L) combined with perampanel (100 nmol/L) caused a significant reduction in seizure activity from 95.6% (confidence interval [CI] 81 to 110) in the absence of DA to 76.8% (CI 63-90, $P = 0.048$) in its presence, and a block at 600 μmol/L. A similar activity is also shown at higher perampanel (500 nmol/L) concentrations, reducing baseline (10 μmol/L) inhibition to 69.6% (CI 53-86, $P = 0.015$). These data also demonstrate a reduced IC_{50} for DA from 352 μmol/L (CI 200-621) to 196 μmol/L (145-264) and 122 μmol/L (49-302, $P = 0.0252$) at 100 and 500 nmol/L, respectively.

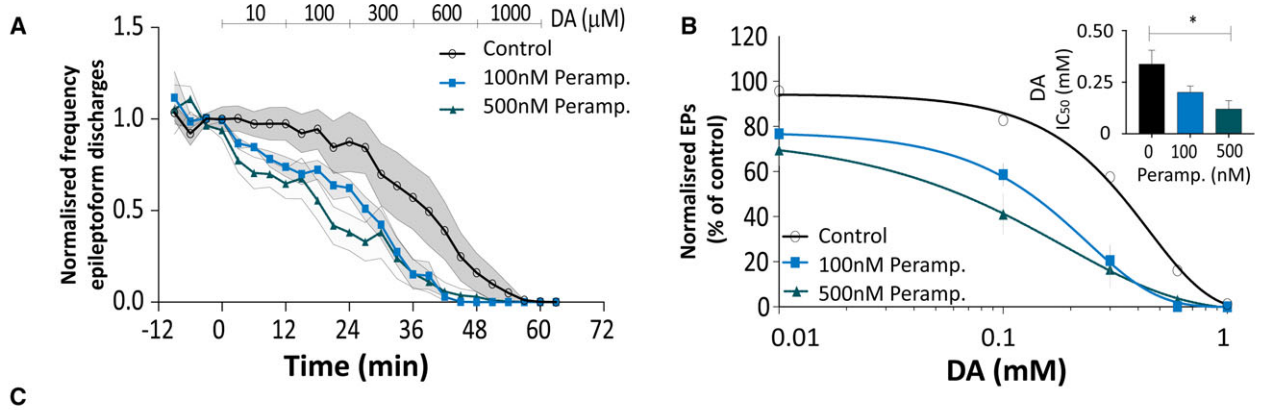
3.3 | Effects of perampanel and decanoic acid on ex vivo human brain slice seizure activity

We then investigated the translational potential of the observed synergism between perampanel and DA in

FIGURE 1 Characterization of direct synergistic inhibition of AMPA receptors by perampanel and decanoic acid (DA). AMPA (GluA2/3, GluA1/2, or GluA3) receptors were expressed in *Xenopus* oocytes, and perfused with L-glutamate (100 μmol/L) and the indicated compound, unless stated otherwise. Currents were recorded using TEVC. A, Representative current traces of inhibitory dose-response curves for DA on GluA2/3 receptors at 1 or 4 μmol/L perampanel. Dose-response inhibition curves for DA at 1 or 4 μmol/L perampanel on (B) GluA2/3 and (C) GluA1/2 receptors. Points were normalized to maximal response to L-glutamate and solvent, 1 μmol/L perampanel, or 4 μmol/L perampanel and represent means and standard error of the mean (SEM) of 8-13 readings. Inserts show respective IC_{50} values in the presence of perampanel. D, Representative current traces of inhibitory dose-response curves for perampanel on GluA2/3 receptors at 50 to 100 μmol/L DA. Dose-response inhibition curves for perampanel at 50 and 100 μmol/L DA on (E) GluA2/3 and (F) GluA1/2 receptors. Points were normalized to maximal response to L-glutamate with solvent, 50 and 100 μmol/L DA and represent means and SEM of 8-13 readings. Inserts show respective perampanel IC_{50} values in the presence of DA. Scale bars correspond to 60 and 75 nA for 1 and 4 μmol/L perampanel, respectively (A) and 200 and 50nA for 50 and 100 μmol/L DA, respectively (D). G, H, Isobolic analysis of the effect of perampanel and DA, showing the isobole (straight line) representing combinatorial doses that are additive. Data points, shown in the line below the isobole, indicate a synergistic effect between each compound at that dose combination *** $P<0.001$; **** $P \leq 0.0001$



reducing epileptiform activity in human brain explants.[10] Slices were obtained from 8 patients undergoing elective neurosurgery for removal of intraaxial neoplasms (Figure 2C). Using a proconvulsant artificial cerebrospinal fluid, epileptiform activity was induced and the impact of DA, perampanel, and combinatory treatment was examined (Figure 2D,E). Baseline epileptiform activity had a mean (95% CI) area power of 1690 μV^2 (CI 1192-2191 μV^2; n = 21). In the presence of DA (100 µmol/L) alone the activity was reduced (1088 μV^2, CI: 393-1782 μV^2; n = 8) but this difference was not statistically significant ($P = 0.1390$). The application of perampanel (0.5 µmol/L)



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Age	Sex	Seizure type	Pathology	AED
54	M	Unwitnessed collapse, incontinence & confusion presumed seizure	Right frontal oligodendroglioma grade II	LEV
50	M	Focal onset seizures with retained awareness	Right amygdala DNET	LEV, LTG
69	M	Focal onset seizures with secondary generalisation	Right temporal glisarcoma grade IV	LEV
24	M	Generalised tonic-clonic seizure	Left termporoparietal lobe grade II astrocytoma	LEV
59	M	Generalised tonic seizure	Left anterior termporal lobe grade IV glioblastoma	LEV
64	M	Nil	Right parietal glioblastoma grade IV	Nil
51	M	Generalised tonic-clonic seizure	Right temporal lobe glioblastoma grade IV	LEV
43	F	Focal onset seizure with impaired awareness	Left temporal glioblastoma grade IV	LEV

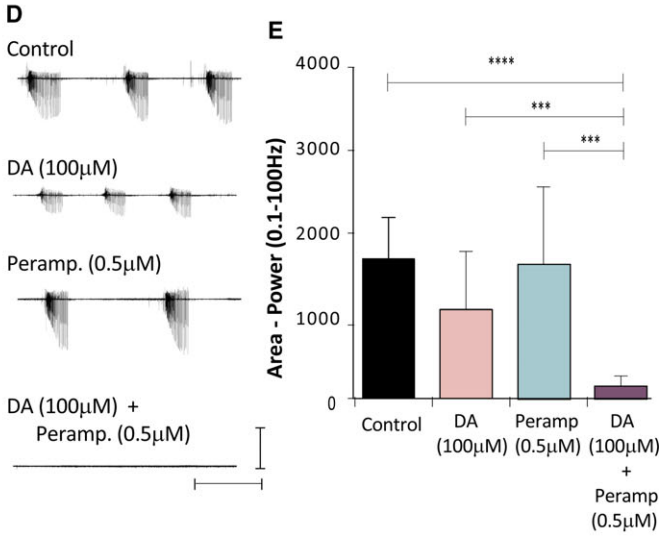

FIGURE 2 Synergistic activity of perampanel and decanoic acid (DA) in rodent and human seizure models. A, Epileptiform (paroxysmal) activity was induced in rat entorhinal cortex-hippocampal slices by application of pentylenetetrazole (PTZ; 2 mmol/L) and $[K^+]$ (to 6 mmol/L) and recorded over time at fixed perampanel and increasing DA concentration. B, Epileptiform activity was normalized to activity in absence or presence of each concentration (100 and 500 nmol/L) and shown at variable DA concentrations. Insert shows DA IC_{50} data for epileptiform activity with perampanel. Data are from 7-8 slices for each group. C, Summary of clinical detail pertaining to tissues used in this study. DNET, dysembryoplastic neuroepithelial tumor; LEV, levetiracetam; LTG, lamotrigine. D, Epileptic activity was induced in human neocortical slices by the perfusion of modified cerebrospinal fluid (0.25 mmol/L Mg^{2+} and 8 mM K^+), and the example traces demonstrate the impact of DA (0.1 mmol/L) alone, perampanel (0.5 mmol/L) alone, and DA and perampanel on pathologic network activity. E, The area power (0.1-100 Hz) of epileptic activity for each of the experimental conditions. Scale bars represent 1 mV and 5 minutes (D) * $P < 0.05$; *** $P < 0.001$; **** $P \leq 0.0001$

alone also did not have a significant effect ($P = 0.7554$) on activity when compared to baseline activity (1621 μV^2, CI: 681-2562 μV^2; n = 10). In contrast, the concurrent application of DA and perampanel significantly ($p < 0.0001$) reduced the area power of epileptiform activity (144.7 μV^2, CI: 15.6-274 μV^2; n = 11) with a virtual abolition of the pathologic network LFP (local field potential) activity. In addition, the combination of compounds had a significantly greater effect when compared with both DA ($P = 0.0005$) and perampanel ($P = 0.0002$) alone.

4 | DISCUSSION

These data confirm that combinatory use of DA and perampanel is effective in directly reducing AMPA receptor generated currents and in reducing PTZ-induced seizure-like activity in hippocampal slice preparations. Furthermore, this synergistic effect is also evident with epileptiform activity generated in resected human neocortical tissue, emphasizing the translational potential for this therapeutic approach in patients with epilepsy.

Mechanistic studies of perampanel inhibition of AMPA receptors have been complicated by low solubility, non-standard concentration response curves, and variable literature. Plasma concentrations of perampanel have been reported to vary between 1.06 and 3.26 μmol/L.[11] Our data indicate that perampanel directly inhibits AMPA receptor currents with IC_{50} values of 5.1-6.2 μmol/L, similar to that shown using native rat and human cerebellar AMPA receptors microtransported into *Xenopus* oocytes (2.6-5.8 μmol/L[12]). Why this is so different from IC_{50} values recorded from rat

neurons (60-93 nmol/L[13,14]) is unclear, but may be due to the presence of other proteins in rat neurons.

Levels of DA have also been monitored in both human and animal models. In the blood of patients on the MCT ketogenic diet, the concentration of DA averages 157 μmol/L.[15,16] In rodent models, the ratio of DA in blood plasma to brain is around 0.7,[17] and extrapolating this to the human brain suggests that DA is likely to be present at around 110 μmol/L. Our data indicate that 100 μmol/L DA results in a 3-fold increase in the inhibition of the AMPA receptor by perampanel, which translates to an even greater impact on seizure activity. In a clinical setting, these observations suggest that, with dietary decanoic acid, a reduction in perampanel dose to, for example, 2-4 mg/d, may provide effective seizure control. These data support the synergistic inhibition of AMPA receptors through combinatory treatment with perampanel and dietary medium chain triglycerides containing DA that may provide both improved seizure control and reduced side effects in patient populations.

In the validation of new approaches for the treatment of epilepsy, in vivo animal studies are often used. The difficulty with in vivo analysis for this study is that metabolism of decanoic acid (or the MCT diet) provides a significant problem because rodents have a much increased metabolic rate (compared to humans). This is likely to lead to the rapid metabolism of decanoic acid in vivo, and thus does not elevate brain levels for a sufficient time following a single dose. Two in vivo studies of medium chain fatty acids in seizure control have used either 10-day MCT ketogenic diets[18] or large single bolus delivery of these fats[17] to analyze brain and blood levels. Due to this rapid metabolic effect, the only way to monitor synergistic effects related to decanoic acid and perampanel using an in vivo rodent model would be to establish IC_{50} values for perampanel, in animals under standard dietary conditions or following maintenance on the MCT diet. However, this approach would not distinguish between potential synergy caused by decanoic acid or the resulting ketones provided by dietary treatment. Thus, since both MCT diets and perampanel are currently used in clinical treatment, future studies would need to investigate therapeutic outcomes in patient groups on an MCT diet with additionally prescribed perampanel.

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DISCLOSURES

RSBW and MW are named inventors on WO2016038379 and WO 2012069790 A1. Funding for KA was provided by Vitaflo Ltd. No other disclosures are reported. 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.

AUTHOR CONTRIBUTIONS

RSBW, MW, PEC, AD, MC, and KA contributed to the conception and design of the study. KA, RSBW, SW, and MC contributed to the acquisition and analysis of data. RW, MW, PC, and KA provided a substantial contribution to drafting the paper. MF, AJ, MAH, DH, PM, and AJ provided human tissue and contributed to writing the manuscript.

DATA AVAILABILITY

All data related to this study is available in the article.

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SUPPORTING INFORMATION

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

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