**MEDIUM CHAIN FATTY ACIDS**

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**Introduction and rationale for development**

Medium chain fatty acids, including decanoic acid, provide the key constituents of the medium chain triglycerides (MCT) in the MCT ketogenic diet. This diet provides one of the last options for the treatment of patients with refractory epilepsy and has been validated in a clinical trial in childhood epilepsy (Augustin et al, 2018). Although often effective, the diet is more commonly used in the treatment of paediatric patients rather than adults, due to the stringent dietary restrictions. The mechanism of the ketogenic diet is broadly considered to be through the hydrolysis of triglycerides to release free fatty acids (decanoic acid and octanoic acid, in the case of the MCT diet), that are metabolised to ketones to provide seizure control (Augustin et al, 2018). This mechanism has recently been challenged, with the proposal that decanoic acid functions as the therapeutic component of the diet (Chang et al 2016), and that this mechanism can be reproduced by specific related congeners.

Decanoic acid was first suggested as the active component of the diet from research in a simple biomedical model (Chang et al, 2012) where a range of novel fatty acids were proposed as seizure control treatments. Several of these fats have been subsequently validated in a range of *in vitro* and *in vivo* models (Chang et al, 2013, 2015). Further analysis of decanoic acid showed that it functions to block activity in two acute *ex vivo* rat hippocampal slice models of epileptiform activity, and this effect was not evident for ketones nor octanoic acid (Chang et al, 2015).

**Pharmacology**

Release of decanoic acid from triglycerides in the gastrointestinal tract enables direct absorption through the gut wall, where it is mostly metabolised in the liver through β-oxidation to produce ketone bodies. However, some fatty acids escape into the circulatory system. In animal models, decanoic acid has been found to penetrate the blood-brain barrier and to be present in brain at 60%– 80% of serum levels (Wlaz et al, 2015). In the brain, decanoic acid functions as a non-competitive inhibitor of the AMPA receptor (Chang et al, 2016), with an IC50 of 0.52±0.02 mM. This is considerably lower than that for octanoic acid (IC50=3.82±0.03 mM).

*Anticonvulsant profile in animal models*

Decanoic acid (1 mM) blocks PTZ- or low magnesium-induced epileptiform discharges in rat entorhinal cortex-hippocampus slices within 20 min of exposure (Chang et al, 2013,2016). The first study to show an antiseizure effect of decanoic acid involved i.p. administration to mice 15 min before seizure induction by s.c. injections of picrotoxin. Decanoic acid caused a small but significant delay in the onset to clonic convulsions at 172 mg/kg (from around 480 s without treatment to around 970 s with decanoic acid) whereas fatty acids with longer chain lengths caused greatly increasing onset time, with palmitic acid (16 carbons) delaying convulsions to around 3600 s, and decanoic acid did not increase survival time (Nakamura et al, 1990). Using the same administration route, this study also suggested that onset to clonic seizures induced by subcutaneous PTZ was not affected by decanoic acid at 172 mg/kg, but survival times increased from around 422 s to 633 s. Although this study suggested a weak effect of decanoic acid on seizure activity, the small effect size and inconsistency between models did not provide strong evidence for an antiseizure effect.

However, two subsequent studies did suggest a role for decanoic acid in seizure control. Using a single bolus oral gavage dose of decanoic acid, seizure thresholds in the 6 Hz model were significantly increased (at 1.7 g/kg and 5.2 g/kg), and a similar increase in the MES threshold was observed at 8.6 g/kg p.o. (Wlaz et al, 2015), although no effect was observed at decanoic acid doses up to at 8.6 g/kg p.o following seizure induction with i.v. PTZ. In addition, dietary intake of 35% of calories through decanoic acid (only) triglycerides increased seizure threshold in the 6 Hz model and the latency to first generalised seizure in the flurothyl model (Tan et al, 2013). These effects were not seen following octanoic acid (only) triglyceride dietary intake. Importantly, this study also demonstrated that both decanoic acid and octanoic acid triglycerides provided a common levels of ketosis, but only the decanoic acid diet provided seizure control, strongly supporting a role for decanoic acid in seizure control independent of ketone generation. In addition to decanoic acid, a range of congeners, including branched and cyclic derivatives, also show strong seizure control activity (Chang et al, 2013, 2015, 2016).

*Other pharmacological properties*

Decanoic acid has been shown to increase mitochondrial proliferation through regulation of a fatty acid receptor, PPARγ (Hughes et al, 2014). In this study, decanoic acid treatment of cultured neural cells was shown to act via PPAR to enhance mitochondrial biogenesis and activity of the mitochondrial complex I. This mechanism was not evident for octanoic acid, and is thought to increase ATP availability leading to an increase in seizure threshold and to a reduction in seizure activity following long term treatment.

*Mechanisms of action*

Studies using whole cell patch clamp recordings from CA1 pyramidal neurons have shown that decanoic acid reduces excitatory postsynaptic currents (EPSCs), consistent with an effect on postsynaptic excitatory AMPA receptors (Chang et al, 2016). Direct inhibitory activity of decanoic acid against AMPA receptors was then shown using a *Xenopus* oocyte model, where the expression of distinct AMPA receptor subunits (GluA1, GluA2 and GluA3) enabled the detailed analysis of decanoic acid-dependent inhibition in isolated receptors (Chang et al, 2016). *In silico* docking analysis suggests decanoic acid binds to AMPA receptors on the M3 helices within the channel region (Chang et al, 2016), providing a distinct binding site to that proposed for perampanel – a currently licensed treatment for focal and generalised tonic-clonic seizures through AMPA receptor inhibition.

Decanoic acid directly inhibits AMPA receptors of different subunit combinations, with greatest potency against GluA2/3 (IC50 = 0.52 mM), followed by GluA1/2 (IC50 = 1.16 mM), and then GluA1 only (IC50 = 2.09 mM). This suggests activity against the most common receptor combinations in the mammalian brain (Chang et al, 2016). Furthermore, the inhibitory effect of decanoic acid against AMPA receptors is voltage-dependent, where potency against GluA2/3 receptors at -80 mV (IC50 of 1.11 mM) is enhanced under more depolarised condition at -40 mV (to IC50 of 0.43 mM), suggesting stronger inhibitory activity during prolonged seizure activity.

**Toxicology**

Few reports of decanoic acid toxicology have been published. Unpublished data kindly provided by the Epilepsy Therapy Screening Program suggests no behavioural toxicity in mice at up to 300 mg/kg (i.p.). High concentration single bolus gavage experiments in mice suggest impaired motor performance in the chimney test with a TD50 of 17.6 g/kg, and no single dose was shown to significantly impair grip strength (Wlaz et al, 2015).

**Pharmacokinetics**

Several early studies have monitored medium chain fats in peripheral blood from children on the MCT ketogenic diet (reviewed by Augustin et al, 2018). These studies show wide variation in decanoic acid levels (87 – 552 μM, with an average of 157 μM). In contrast, patients had greater levels of octanoic acid, averaging 310 μM, a finding which may reflect the higher content of octanoic acid provided within the MCT supplement. Although octanoic acid is unlikely to inhibit AMPA receptors at therapeutic concentrations, it may function to elevate neuronal decanoic acid levels through preferential oxidation (Augustin et al, 2018).

**Drug interactions**

Contraindications for the ketogenic diet include defects in fatty acid metabolism and function including fatty acid oxidation, and deficiencies in carnitine-related function, organic translocase, pyruvate carboxylase and hypoglycaemia.

**Efficacy data**

A randomized trial suggested that the classical and the MCT ketogenic diets may show similar efficacy in seizure control, although the classical diet requires a more stringent regimen (Neal et al, 2009). In that trial, the classical diet and the MCT diet were administered to a total of 125 children with pharmacoresistant epilepsy, but only 47 patients were evaluable for efficacy at the 12 month assessment. The average percent decrease in seizure frequency compared with baseline was 53% for the evaluable children in the MCT ketogenic diet group (n=25) and 40.8% for those evaluable in the classical ketogenic diet group (n=22). No breakdown of outcome by seizure type was reported.

**Tolerability and side effect profile**

Although no data exist regarding decanoic acid-only treatment in patient groups, the MCT ketogenic diet is associated with a range of gastrointestinal-related side effects, such as cramps, bloating, diarrhoea, and vomiting. In addition, it is important to note that AMPA receptors play an important role in synaptic strengthening during long-term potentiation and synaptic plasticity. However, inhibition of AMPA receptor activity has been demonstrated not to impair long-term potentiation or cognition (for review, see Chang et al, 2016), suggesting that dietary decanoic acid is unlikely to adversely affect learning and memory. In contrast, the MCT ketogenic diet has been reported to be associated with positive effects on cognition (Taylor et al 2018) although the mechanism of this positive effect remains to be identified.

**Planned studies**

A recently developed modified MCT diet containing high levels of decanoic acid (Betashot) is currently in tolerability clinical trials (tolerability) with both adult and paediatric drug-resistant patients (NCT02825745). This study involves less stringent dietary restrictions than the currently used MCT ketogenic diet.

**Disclosure of conflict of interest**

Mathew Walker and Robin Williams have received research funding from Vitaflo Ltd, have received Consultancy and/or Speakers’ fees from UCB pharma, and hold a patent (WO 2012069790, WO 2013186570) related to this work.

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**Table 1: Anticonvulsant profile1 and proposed mechanism(s) of action**

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Route of**  **Administ-ration** | **Time of**  **Test (min)** | **MES** | **scPTZ** | **6Hz 22mA** | **6 HZ**  **32 mA** | **6 Hz 44mA** | **Corneal**  **Kindled mouse** | **Kindled rat**  **(i.e., amygdala, hippocampal)** | **Spike-wave seizure model**  **2** | **Behavioral Toxicity**  **(e.g.,rotarodobservational etc)** | **In vivo activity in other disease model systems actions3** | **Proposed mechanism(s) of action** |
| Mouse | i.p. of decanoic acid7 | 15 | NT | 86 and 172mg/kg small but significant increase in survival time | NT | NT | NT | NT | NT | NT | NT | NT | NT |
|  | i.p. 100mg/kg | 15-240 | NT | NT | NT | No control | NT | NT | NT | NT | NT | NT | NT |
|  | i.p. up to 300mg/kg | 120 | NT | NT | NT | NT | No control | NT | NT | NT | NT | NT | NT |
|  | p.o.  bolus gavage of decanoic acid6 | 30 | significant increase in CS50 (mA) at 8.6g/kg | NT | NT | significant increase in CS50 (mA) at 1.7g/kg and 5.2g/kg | NT | NT | NT | NT | chimney test motor-impairing TD50 value 17.6 g/kg | NT | NT |
|  | p.o. 10- day dietary intake of decanoic acid triglyceride (35% calories)8 | N/A | NT | NT | NT | Significant increase in CC50 (mA) | NT | NT | NT | NT | NT | NT | NT |
|  | i.v. |  |  |  |  |  |  |  |  |  |  |  |  |

**1 Please report results (where available) as median effective (ED50) or median toxic (TD50) in mg/kg. If an ED50 could not be determined because of a lack of efficacy, please indicate the results obtained at the maximum dose tested; e.g., maximum 50% protection at a dose of 100 mg/kg.**

**2 If appropriate please define which spike-wave model was employed.**

**3 Please provide any relevant summary of demonstrated activity in other disease models; i.e., pain, migraine, bipolar, etc. Where appropriate, please provide sufficient experimental detail of study design and methods employed.**

**4 If results are not available for one or more seizure tests because compound has not been tested, please indicate by placing NT in the appropriate column.**

**N/A, not applicable**