EVALUATION OF SYNTHETIC DERIVATIVES OF MEDIUM CHAIN TRIGLYCERIDE (MCT) FATTY ACIDS IN MICE

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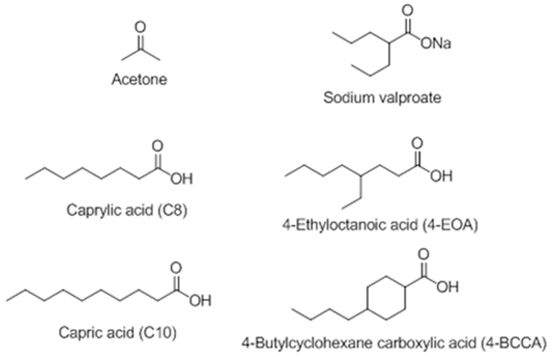
Introduction

The MCT ketogenic (KG) diet is considered among the most effective therapies for certain refractory epilepsies and has been proposed to be of value for treatment of pain and inflammatory conditions. The MCT KG diet contains medium chain fatty acids (MCFA) with caprylic acid (C8) and capric acid (C10) being primary constituents. Acute treatment with C8 or C10 increases blood levels of ß-hydroxybutyrate (ßHB) - a biomarker of ketosis, but both compounds only show very modest effects in mouse seizure models compared with synthetic anti-epileptic drugs (AED's) (e.g. Wlaz et al (2012) Neuropharmacol. 62: 1882-1889). Recently Chang et al (2015; JPET 352: 43-52) described a novel series of synthetic derivatives of MCFA (e.g. 4-EOA and 4-BCCA) with evidence for improved tolerability and efficacy compared to C8 and C10, and thus a possible improvement over the MCT KG diet. The present studies benchmarked a selection of these synthetic derivatives to C8, C10 and acetone. Valproate was included for comparison as a structurally related AED.



Methods

Male CD-1 mice (source: Charles River, St. Constant, Quebec, Canada) were used for all tests.

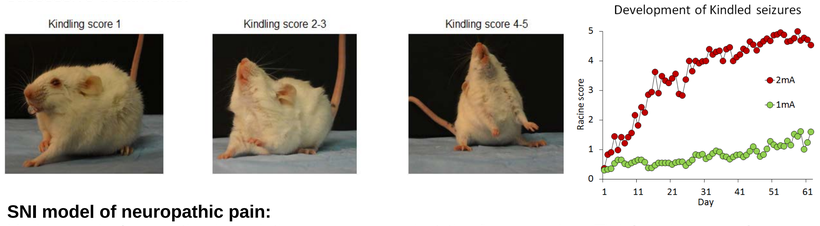
Seizure tests:

Mouse MES assay: Following a defined drug pretreatment, mice received a maximal electroshock (45mA, 0.2s duration, 60Hz) via corneal electrodes moistened with saline. Protection defined as absence of a full tonic seizure within 10s of stimulus delivery. In some studies an MES threshold test (MES-T) was conducted using up-and-down method. Threshold approximately 10mA.

Mouse s.c PTZ assay: Following a defined drug pretreatment all mice received a single subcutaneous injection of pentylenetetrazol (PTZ; 85mg/kg). The animals were then transferred to single observation cages and observed continuously for 30min. Protection defined as complete absence of a clonic seizure over the 30min observation period. In the event of a seizure, the onset latency from PTZ injection was recorded.

Mouse 6Hz assay: Following a defined drug pretreatment, all mice received an electrical stimulus (6Hz, 0.2ms pulse width, 3s duration, 32mA) via corneal electrodes. A psychomotor seizure, was defined as the expression of at least one of the following behaviours: stun/immobility, forelimb clonus, straub tail, vibrissae tremor, lateral head movement in >95% of control animals. Protection was defined as complete absence of all the above behaviours within 20s of stimulus delivery.

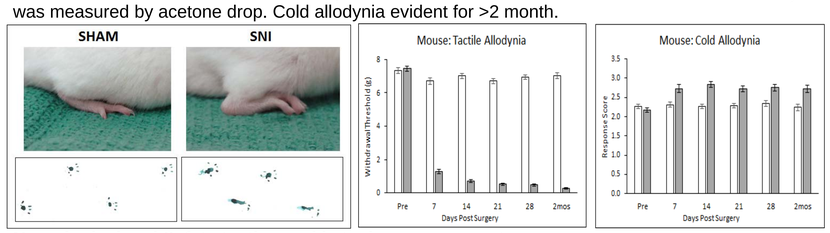
Mouse corneal kindling assay: Male, CD-1 mice received a mild electrical stimulus 2 x daily (2mA, 60Hz, 3s duration). via corneal electrodes moistened with saline. This stimulus intensity did not initially elicit seizures, rather a mild behavioural response, e.g brief (<5s) immobility, stare. Mice received 2 such stimulations per day. Over approximately 15 days, the animals developed transient behavioural changes typified by brief motor seizures for approximately 30s following stimulation. The progressive behavioural changes are rated according to a scale developed by Racine (1972) for rat, i.e. 0 = no effect; 1 = jaw clonus; 2 = myoclonic twitches forelimbs, head nodding; 3 = forelimb clonus; 4 = forelimb clonus with rearing; 5 = clonus with rearing and loss of balance. Drug testing was conducted according to a repeated measures design with at least 2 drug free days between successive treatments.



SNI model of neuropathic pain:

Under surgical anaesthesia, sciatic nerve exposed at the femur region. Tibial and peroneal nerves transected keeping sural nerve intact. Following closure of the wound, animals allowed to recover for 20 days pre-testing. On specific occasions, each mouse tested for mechanical and cold allodynia. Drug testing conducted to a repeated measures design with ≥2 drug free days between successive treatments – Pregabalin (PGB, 30 mg/kg 2h ptt.) included as a reference control in each experiment.

Mechanical allodynia was measured using Von Frey hairs. The threshold was taken as the lowest force that evoked a brisk withdrawal response. Tactile allodynia evident for >2 month. Cold allodynia was measured by acetone drop. Cold allodynia evident for >2 month.



Neurological and Safety testing and ketosis measurement:

At multiple timepoints post NCE treatment (pre, 1h, 2h, 4h, 6h), mice were assessed for core body temperature and blood ßHB levels (measured using a commercial ßHB meter) and/or urinary acetoacetate. Locomotor activity and rotorod performance (16 r.p.m. and 32 r.p.m.) was measured at 40min – 1h post treatment. Three day safety study also conducted for 4-EOA.

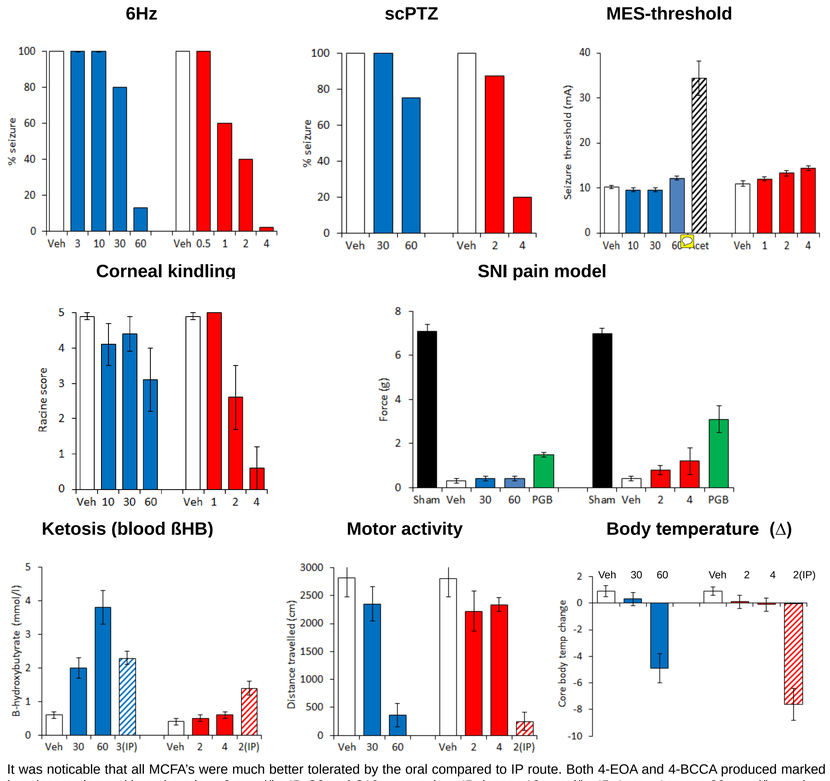
Results

Summary Data:

		6Hz		MES		scPTZ		MES-T	Corneal Kindling		SNI (NP pain)	Neurological		Ketosis
		IP	oral	IP	oral	IP	oral	oral	IP	oral	oral	LMA	BT	BHB (blood)
Acetone	mg/kg	620	1250	1250	2500	1850	2500	1000	1250	1500	>3700	++	+	++
	mmol/kg	10	20	20	40	32	40	16	20	25	>64	64 (oral)	64 (oral)	64 (IP)
Caprylic acid	mg/kg	1440	7200	>1440	>8650	1440	>8650	10,000	NT	8650	>8650	++	+	+++
	mmol/kg	10	50	>10	>60	10	>60	60	NT	60	>60	60 (oral)	60 (oral)	60 (oral)
Capric acid	mg/kg	10,000	>10,000	>5,000	>10,000	3,500	>10,000	>10,000	NT	>10,000	>10,000	+	+	+
	mmol/kg	60	>60	>30	>60	20	>60	>60	NT	>60	>60	60 (oral)	60 (oral)	60 (oral)
4-EOA	mg/kg	150	200	200	900	200	500	300	200	300	600	0	0	++
	mmol/kg	0.9	1.2	1.2	5.4	1.2	3	1.8	1.2	1.7	4	4 (oral)	4 (oral)	2 (IP)
4-BCCA	mg/kg	100	200	200	>600	175	400	NT	200	300	>300	0	0	++
	mmol/kg	0.5	1.1	1.1	>4	1	2.2	NT	1.1	1.6	>2	2 (oral)	2 (oral)	2 (IP)
Sodium Valproate	mg/kg	NT	200	NT	244	NT	300	300	NT	300	300	0	0	0
	mmol/kg	NT	1.2	NT	1.5	NT	1.9	1.9	NT	1.9	1.9	4 (oral)	4 (oral)	4 (oral/IP)

Data is presented as ED_{50} where active, or if inactive a greater than (>) dose is presented. NT = not tested. Doses expressed as mg/kg or mmol/kg to enable comparisons between MCFA's. LMA = motor activity, BT = core body temperature. 0 = no change, + = modest hypoLMA or hypothermia, ++ = marked change. ßHB measure (peak) 0 = no change, + = modest (0.6-1 mmol/l), ++ (1-2 mmol/l), +++ (>2 mmol/l).

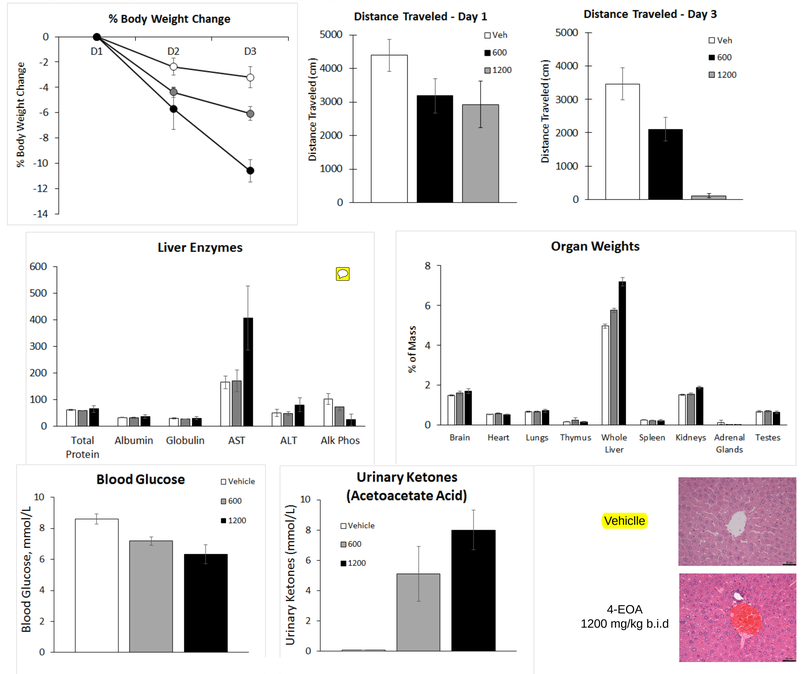
Comparison between Caprylic acid and 4-EOA: (oral, unless otherwise stated)



It was noticable that all MCFA's were much better tolerated by the oral compared to IP route. Both 4-EOA and 4-BCCA produced marked hypolocomotion and hypothermia at 2 mmol/kg IP. C8 and C10 were toxic at IP doses ≥10 mmol/kg IP. Acet = Acetone 32 mmol/kg oral.

Results (cont.)

Safety (3 day 4-EOA 600 mg/kg & 1200 mg/kg b.i.d treatment):



Male CD-1 mice were dosed 2 x daily for 3 days with either vehicle, 4-EOA (600 mg/kg or 1200 mg/kg b.i.d; approx. equivalent to 4 and 8 mmol/kg b.i.d). Neurological tests were conducted 1h post treatment on D1 and D3. Single treatment with 4-EOA at both doses produced hypoglycaemia and ketosis (urinary acetoacetic acid). Neurological function was unimpaired. However by D3, mice treated with 4-EOA at 1200 mg/kg b.i.d developed marked weight loss, hypolocomotion and signs of liver steatosis. Hypoglycaemia and urinary ketosis was also evident in both treatment groups.

Summary and conclusions

1. **Rank order of activity across seizure tests:- EOA=BCCA=Valproate>Acetone>C8>C10.**
2. **4-EOA and 4-BCCA showed a broad spectrum of efficacy across multiple seizure tests, and both well tolerated at efficacious doses of 1-4 mmol/kg when administered by the oral route, (i.e motor/neurological function normal).**
3. **All compounds, except VPA were significantly more potent by IP route. Minimal separation between efficacy and side-effect for 4-EOA, 4-BCCA, Acetone, C8, C10 by IP route.**
4. **Anti-seizure effects of C8 and C10 were considered marginal, even at doses that were poorly tolerated. Acetone has significantly broader efficacy vs. C8/C10 e.g. MES test.**
5. **Relationship between ketosis and ant-seizure efficacy is unclear. There appears to be no relationship between ketosis and AED efficacy, although biomarkers of ketosis limited to plasma [ßHB]. Urinary acetoacetate may be a better biomarker for ketosis.**
6. **The 6Hz test seems most sensitive seizure assay to each drug and the test has also been reported as sensitive to KTG diet (Hartman et al (2007) Pediatr. Neurol. 36: 281-292).**
7. **Only v. modest effect of 4-EOA (and 4-BCCA) against tactile and cold allodynia in SNI model. Effect size significantly less that of PGB.**
8. **Studies support further research into synthetic MCFA derivatives as treatments for epilepsy. Both 4-EOA and 4-BCCA may represent an advance on the MCT KG diet for the treatment of refractory epilepsy, having a broad spectrum profile and potency similar to VPA (2-4 mmol/kg oral). Efficacy profile compares favourably to marketed AED's.**

	Mouse						Rat	
	MES (45mA)	MES-T	scPTZ	6Hz	Kindling (corneal)	MES (150mA)	scPTZ	Kindling (amygdala)
Acetone	+	+	+	+	+	+	NT	+
4-EOA	+	+ (partial)	+	+	+	NT	NT	NT
4-BCCA	+	NT	+	+	+	NT	NT	NT
Carbamazepine (Tegretol®)	+	+	-	+	+	+	NT	+
Na Valproate (Depakote®)	+	+	+	+	+	+	+	+
Lacosamide (Vimpat®)	+	+	-	+	+	+	-	+
Pregabalin (Lyrica®)	-	+	NT	+	+/-	+	-	+
Phenytoin (Dilantin®)	+	+	-	-	+	+	-	+/-
Retigabine (Ezogabine®)	+ (high)	NT	+ (high)	+	NT	NT	NT	NT
Diazepam (Valium®)	+ (high)	NT	+	+	NT	+	+	NT